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(54) Title: MODULATING DEVELOPMENTAL PATHWAYS IN PLANTS

(57) Abstract: The invention relates to a method to modulate plant growth or development by modifying genes in plants. The invention among others relates to modifying RKS genes or gene products as found in *Arabidopsis thaliana* or other plants. The invention provides a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein.

Title: Modulating developmental pathways in plants.

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The invention relates to a method to modulate plant growth or development by modifying genes in plants. The invention among others relates to modifying RKS genes or gene products as found in *Arabidopsis thaliana* or other plants. The 10 different domains of RKS gene products essentially have the following functions: The first domain of the predicted protein structure at the N-terminal end consists of a signal sequence, involved in targeting the protein towards the plasma membrane. Protein cleavage removes this sequence from the final mature 15 protein product (Jain et al. 1994, J. Biol. Chemistry 269: 16306-16310). The second domain consists of different numbers of leucine zipper motifs, and is likely to be involved in protein protein dimerization. The next domain contains a conserved pair of cystein residues, involved in disulphate 20 bridge formation. The next domain consists of 5 (or in the case of RKS3 only 4) leucine rich repeats (LRRs) shown in a gray colour, likely to be involved in ligand binding (Kobe and Deisenhofer 1994, TIBS 19: 415-420). This domain is again bordered by a domain containing a conserved pair of cystein 25 residues involved in disulphate bridge formation often followed by a serine / proline rich region. The next domain displays all the characteristics of a single transmembrane domain. At the predicted cytoplasmic site of protein a domain is situated with unknown function, followed by a domain with 30 serine /threonine kinase activity (Schmidt et al. 1997, Development 124: 2049-2062, WO 01/29240). The kinase domain is followed by a domain with unknown function whereas at the C-terminal end of the protein part of a leucine rich repeat is positioned, probably involved in protein-protein interactions.

35

Plant homologs of the *Arabidopsis* RKS genes can be found by comparison of various plant database (see also Table 2) and comprise amongst others:

5 Y14600 | SBRLK1 | *Sorghum bicolor*
BF004020 | BF004020 | EST432518 KV1 *Medicago truncatata*
AW934655 | AW934655 | EST353547 tomato
AW617954 | AW617954 | EST314028 *L. pennellii*
AA738544 | AA738544 | SbRLK2 *Sorghum bicolor*

10 AA738545 | AA738545 | SbRLK3 *Sorghum bicolor*
BG595415 | BG595415 | EST494093 cSTS *Solanum tuberosa*
AI896277 | AI896277 | EST265720 tomato
BF643238 | BF643238 | NF002H05EC1F1045
AA738546 | AA738546 | SbRLK4 *Sorghum bicolor*

15 BE658174 | BE658174 | GM700005A20D5 Gm-r1070 *Glycine max*
BF520845 | BF520845 | EST458318 DSIL *Medicago truncata*
AC069324 | AC069324 | *Oryza sativa*
AW761055 | AW761055 | sl70d06.y1 Gm-c1027 *Glycine max*
BE352622 | BE352622 | WHE0425_G11_M21ZS Wheat

20 BG647340 | BG647340 | EST508959 HOGA *Medicago truncata*
AY028699 | AY028699 | *Brassica napus*
AW666082 | AW666082 | sk31h04.y1 Gm-c1028 *Glycine max*
AA738547 | AA738547 | SbRLK5 *Sorghum bicolor*
BG127658 | BG127658 | EST473220 tomato

25 L27821 | RICPRKI | *Oryza sativa*
BG238468 | BG238468 | sab51a09.y1 Gm-c1043 *Glycine max*
BG441204 | BG441204 | GA_Ea0012C15f *Gossypium arbo.*
AW667985 | AW667985 | GA_Ea0012C15 *Gossypium arbore.*
AW233982 | AW233982 | sf32g05.y1 Gm-c1028 *Glycine max*

30 AP003235 | AP003235 | *Oryza sativa*
BF460294 | BF460294 | 074A05 Mature tuber
AY007545 | AY007545 | *Brassica napus*
AC087544 | AC087544 | *Oryza sativa*
AB041503 | AB041503 | *Populus nigra*

35

The invention furthermore relates to modifying ELS genes or gene products or functional equivalents thereof which are for example derived from at least two different genes in the *Arabidopsis* genome. They show high homology on protein level

with the corresponding transmembrane RKS gene products. However, they lack a transmembrane domain while they do contain a signaling sequence at the N-terminal end. Therefore these proteins are thought to be positioned within vesicles 5 within the plant cell or at the outside of the plasma membrane, within the cell wall of the plant cell. A number of homologs have been detected in other plant species, such as:

AF370543|AF370543|*Arabidopsis thaliana*
10 AF324989|AF324989|*Arabidopsis thaliana*
AV520367|AV520367|*Arabidopsis thaliana*
AV553051|AV553051|*Arabidopsis thaliana*
BF642233|BF642233|NF050C09IN1F1069
AW559436|AW559436|EST314484 DSIR *Medicago truncata*
15 BG456991|BG456991|NF099F02PL1F1025
AW622146|AW622146|EST312944 tomato
BF260895|BF260895|HVSMEf0023D15f *Hordeum vulgare*
BE322325|BE322325|NF022E12IN1F1088
BG414774|BG414774|HVSMEk0003K21f *Hordeum vulgare*
20 BE460627|BE460627|EST412046 tomato
BI204894|BI204894|EST522934 cTOS *Lycopersicon esculentum*
BI205306|BI205306|EST523346 cTOS *Lycopersicon esculentum*
BI204366|BI204366|EST522406 cTOS *Lycopersicon esculentum*
AW443205|AW443205|EST308135 tomato
25 AW031110|AW031110|EST274417 tomato
BI180080|BI180080|EST521025 cSTE *Solanum tuberosa*
BF644761|BF644761|NF015A11EC1F1084
AV526127|AV526127|*Arabidopsis thaliana*
AV556193|AV556193|*Arabidopsis thaliana*
30 BE203316|BE203316|EST403338 KV1 *Medicago truncatata*.
AW649615|AW649615|EST328069 tomato
BE512465|BE512465|946071E06
BI204917|BI204917|EST522957 cTOS *Lycopersicon esculentum*
BG590749|BG590749|EST498591
35 BG648725|BG648725|EST510344 HOGA *Medicago truncata*
BG648619|BG648619|EST510238 HOGA *Medicago truncata*
BG597757|BG597757|EST496435 cSTS *Solanum tuberosa*
AW221939|AW221939|EST298750 tomato
BE704836|BE704836|Sc01_
40 BG124409|BG124409|EST470055 tomato

BF051954 | BF051954 | EST437120 tomato
BG320355 | BG320355 | Zm03_05h01_Zea mays
AV526624 | AV526624 | Arabidopsis thaliana
AW933960 | AW933960 | EST359803 tomato

5 AW221278 | AW221278 | EST297747 tomato
BE405514 | BE405514 | WHE1212_C01_F02ZS Wheat
BG314461 | BG314461 | WHE2495_A12_A23ZS Triticum
BF258673 | BF258673 | HVSMEf0016G01f Hordeum vulgare
BG262637 | BG262637 | WHE0938_E03_I06ZS Wheat

10 AW030188 | AW030188 | EST273443 tomato
BG653580 | BG653580 | sad76b11.y1 Gm-c1051 Glycine max
BG319729 | BG319729 | Zm03_05h01_A Zm03_Zea mays
BF053590 | BF053590 | EST438820 potato
BE454808 | BE454808 | HVSMEh0095C03f Hordeum vulgare

15 BI075801 | BI075801 | IP1_21_D05.b1_A002
BE367593 | BE367593 | PI1_9_F02.b1_A002 Sorghum bicolor
2e-074 BF260080 | BF260080 | HVSMEf0021A22f Hordeum vulgare
BF627921 | BF627921 | HVSMEb0006I23f Hordeum vulgare
BG598491 | BG598491 | EST503391 cSTS Solanum tuberosa

20 AW038168 | AW038168 | EST279825 tomato
BG343258 | BG343258 | HVSMEg0005D23f Hordeum vulgare
AW925684 | AW925684 | HVSMEg0005D23 Hordeum vulgare
BG416093 | BG416093 | HVSMEk0009L18f Hordeum vulgare
AW683370 | AW683370 | NF011C09LF1F1069

25 BE420108 | BE420108 | WWS020.C1R000101 ITEC WWS Wheat
AW350720 | AW350720 | GM210009A10F4 Gm-r1021 Glycine max
AW616564 | AW616564 | EST322975 L. Hirsutum trichome
AW011134 | AW011134 | ST17B03 Pine
BF630746 | BF630746 | HVSMEb0013N06f Hordeum vulgare

30 AW926045 | AW926045 | HVSMEg0006C10 Hordeum vulgare
BE519800 | BE519800 | HV_CEb0021E12f Hordeum vulgare
BG343657 | BG343657 | HVSMEg0006C10f Hordeum vulgare
BG933682 | BG933682 | OV1_16_C09.b1_A002
BE433368 | BE433368 | EST399897 tomato

35 AW219797 | AW219797 | EST302279 tomato
BF629324 | BF629324 | HVSMEb0010N06f Hordeum vulgare
BE597128 | BE597128 | PI1_71_A07.g1_A002
AW220075 | AW220075 | EST302558 tomato
AW616639 | AW616639 | EST323050 L. Hirsutum trichome

40 BF645214 | BF645214 | NF032F11EC1F1094
AW924540 | AW924540 | WS1_70_H12.b1_A002

AI775448 | AI775448 | EST256548 tomato
AW983360 | AW983360 | HVSMEg0010F15f *Hordeum vulgare*
BF270171 | BF270171 | GA_Eb0007B13f *Gossypium arbor.*
BE919631 | BE919631 | EST423400 potato

5 AW037836 | AW037836 | EST279465 tomato
BF008781 | BF008781 | ss79h09.y1 Gm-c1064 *Glycine max*
BF254651 | BF254651 | HVSMEf0004K05f *Hordeum vulgare*
BE599797 | BE599797 | PI1_79_H01.g1_A002
BE599026 | BE599026 | PI1_86_E03.g1_A002

10 R89998 | R89998 | 16353 Lambda-PRL2 *Arabidopsis*
BG841108 | BG841108 | MEST15-G02.T3 ISUM4-TN *Zea mays*
AW307218 | AW307218 | sf54c07.y1 Gm-c1009 *Glycine max*
AI496325 | AI496325 | sb05c09.y1 Gm-c1004 *Glycine max*
AJ277703 | ZMA277703 | *Zea mays*

15 AL375586 | CNS0616P | *Medicago truncatula* EST
AW350549 | AW350549 | GM210009A10A12 Gm-r1021 *Glycine max*
BE125918 | BE125918 | DG1_59_F02.b1_A002
BF053901 | BF053901 | EST439131 potato
BE921389 | BE921389 | EST425266 potato
20 BE597551 | BE597551 | PI1_71_A07.b1_
BE360092 | BE360092 | DG1_61_C09.b1_A002
BE660084 | BE660084 | 491 GmaxSC *Glycine max*
AJ277702 | ZMA277702 | *Zea mays*

25 The invention also relates to modifying SBP/SPL gene or products which represent a family of transcription factors with a bipartite nuclear localization signal (The SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE (SBP/SPL) gene family of *Arabidopsis thaliana*, Columbia ecotype). Upon activation 30 (probably by RKS mediated phosphorylation, the bipartite nuclear localization signal becomes linear and available for the nuclear translocation of the protein. Within the plant nucleus, the transcription factor regulates transcription by interaction with specific promoter elements. In *Arabidopsis thaliana*, this family is represented by at least 16 different members (see following list). In many other plant species, we 35 also identified members of this transcription factor family (See list on page 7).

Functional interaction between RKS and SBP proteins was shown by studies in transgenic tobacco plants in which SBP5 and RKS0 were both overexpressed under the control of an enhanced 35S promoter (data not shown). At the tip of double overexpressing plants, embryo structures appeared whereas in the SBP5 overexpressing plants alone or the RKS0 overexpressing plants alone no phenotype was detectable at the root tips of transgenic tobacco plants. These results show that both RKS and SBP proteins are involved together in a signalling cascade, resulting in the reprogramming of developmental fate of a determined meristem. (ref. dissertation: <http://www.ub.uni-koeln.de/ediss/archiv/2001/11w1204.pdf>; Plant Journal 1997: 12, 2 367-377; Mol. Gen. Genet. 1996: 250, 7-16; Gene 1999, 237, 91-104, Genes and Development 1997: 11, 616-628), Proc. Natl. Acad. Sci. USA 1998: 95, 10306-10311; The Plant Journal 2000: 22, 523-529; Science 1997: 278, 1963-1965; Plant Physiol. Biochem. 2000: 38, 789-796; Cell 1996: 84, 61-71; Annu. Rev. Plant Physiol. Plant Mol. Biol. 1999: 50, 505-537

20

	name	genetic code
	ATSPL1	At2g47070*
	ATSPL2	At5g43270
	ATSPL3	At2g33810*
25	ATSPL4	At1g53160*
	ATSPL5	At3g15270
	ATSPL6	At1g69170
	ATSPL7	At5g18830
	ATSPL8	At1g02065
30	ATSPL9	At2g42200*
	ATSPL10	At1g27370*
	ATSPL11	At1g27360*
	ATSPL12	At3g60030
	ATSPL13	At5g50570
35	ATSPL14	At1g20980
	ATSPL15	At3g57920
	ATSPL16	At1g76580

* annotation in database not complete and/or correct

In many other plant species, we identified members of this transcription factor family, plant homologs of the *Arabidopsis* SBP/SPL proteins are for example:

5 AB023037 | AB023037 | *Arabidopsis thaliana*
BG789832 | BG789832 | sae56b07.y1 Gm-c1051 *Glycine max*
BG123992 | BG123992 | EST469638 *tomato*
BG595750 | BG595750 | EST494428 cSTS *Solanum tuberosum*
AF370612 | AF370612 | *Arabidopsis thaliana*

10 BF728335 | BF728335 | 1000060H02.x1 1000 - *Zea mays*
X92079 | AMSBP2 | *A. majus*
AW331087 | AW331087 | 707047A12.x1 707 - Mixed adult... 128 zea mays
AJ011643 | ATH011643 | *Arabidopsis thaliana*
L34039 | RICRMSOA | *Oryza sativa*

15 AJ011638 | ATH011638 | *Arabidopsis thaliana*
AJ011639 | ATH011639 | *Arabidopsis thaliana*
AJ132096 | ATH132096 | *Arabidopsis thaliana*
BF482644 | BF482644 | WHE2301-2304_A21_A21ZS *Wheat*
BF202242 | BF202242 | WHE0984_D01_G02ZS *Wheat*

20 BE057470 | BE057470 | sm58e10.y1 Gm-c1028 *Glycine max*
AJ011628 | ATH011628 | *Arabidopsis thaliana*
AJ011629 | ATH011629 | *Arabidopsis thaliana*
AJ011617 | ZMA011617 | *Zea mays*
AJ011637 | ATH011637 | *Arabidopsis thaliana*

25 AJ011622 | AMA011622 | *Antirrhinum majus*
AJ011621 | AMA011621 | *Antirrhinum majus*
AJ011635 | ATH011635 | *Arabidopsis thaliana*
AJ011623 | AMA011623 | *Antirrhinum majus*
BF650908 | BF650908 | NF098D09EC1F1076

30 AJ242959 | ATH242959 | *Arabidopsis thaliana*
Y09427 | ATSPL3 | *A. thaliana* mRNA
AJ011633 | ATH011633 | *Arabidopsis thaliana*
AW691786 | AW691786 | NF044B06ST1F1000
BE058432 | BE058432 | sn16a06.y1 Gm-c1016 *Glycine max*

35 AW728623 | AW728623 | GA_Ea0017G06 *Gossypium arbore.*
BG442540 | BG442540 | GA_Ea0017G06f *Gossypium arbo.*
AJ011626 | ATH011626 | *Arabidopsis thaliana*
AJ011625 | ATH011625 | *Arabidopsis thaliana*
AI993858 | AI993858 | 701515182 *A. thaliana*

40 BG593787 | BG593787 | EST492465 cSTS *Solanum tuberosum*
BF634536 | BF634536 | NF060C08DT1F1065 *Drought Medicago*

BE806499 | BE806499 | ss59f10.y1 Gm-c1062 *Glycine max*
AW933950 | AW933950 | EST359793 tomato
AC008262 | AC008262 | *Arabidopsis*
 B28493 | B28493 | T10A24TF TAMU *Arabidopsis thaliana*
5 AJ011644 | ATH011644 | *Arabidopsis thaliana*
 AC018364 | AC018364 | *Arabidopsis thaliana*
 AL092429 | CNS00VLB | *Arabidopsis thaliana*
 BE435668 | BE435668 | EST406746 tomato
 BG097153 | BG097153 | EST461672 potato
10 BE440574 | BE440574 | sp47b09.y1 Gm-c1043 *Glycine max*
 AI443033 | AI443033 | sa31a08.y1 Gm-c1004 *Glycine max*
 U89496 | ZMU89496 | *Zea mays* liguleless1
 AW433271 | AW433271 | sh54g07.y1 Gm-c1015 *Glycine max*
 AW932595 | AW932595 | EST358438 tomato
15 AW096676 | AW096676 | EST289856 tomato
 AJ011616 | ZMA011616 | *Zea mays*
 AW036750 | AW036750 | EST252139 tomato
 BF626329 | BF626329 | HVSMEA0018F24f *Hordeum vulgare*
 AJ011614 | ZMA011614 | *Zea mays*
20 AJ011642 | ATH011642 | *Arabidopsis thaliana*
 BE022435 | BE022435 | sm85h04.y1 Gm-c1015 *Glycine max*
 X92369 | AMSPB1 | *A. majus*
 AC015450 | AC015450 | *Arabidopsis thaliana*
 AC079692 | AC079692 | *Arabidopsis thaliana*
25 AJ011632 | ATH011632 | *Arabidopsis thaliana*
 AJ011631 | ATH011631 | *Arabidopsis thaliana*
 BE455349 | BE455349 | HVSMEh0097E20f *Hordeum vulgare*
 AJ242960 | ATH242960 | *Arabidopsis thaliana*
 AJ011610 | ATH011610 | *Arabidopsis thaliana*
30 AJ132097 | ATH132097 | *Arabidopsis thaliana*
 AL138658 | ATT209 | *Arabidopsis thaliana*
 AJ011615 | ZMA011615 | *Zea mays*
 BE499739 | BE499739 | WHE0975_ Wheat
 AW398794 | AW398794 | EST309294 *L. pennellii*
35 AJ011618 | ZMA011618 | *Zea mays*
 AW747167 | AW747167 | WS1_66_F11.b1_
 AJ011577 | ATH011577 | *Arabidopsis thaliana*
 AI992727 | AI992727 | 701493410 *A. thaliana*
 BE060783 | BE060783 | HVSMEg0013F15f *Hordeum vulgare*
40 BE804992 | BE804992 | ss34h10.y1 Gm-c1061 *Glycine max*
 BE325341 | BE325341 | NF120H09ST1F1009

AC007369 | AC007369 | *Arabidopsis thaliana*

AJ011619 | ZMA011619 | *Zea mays*

BI099345 | BI099345 | IP1_37_H10.b1_A002

BI071295 | BI071295 | C054P79U *Populus*

5 AZ920400 | AZ920400 | 1006019G01.y2 1006 -

AZ919034 | AZ919034 | 1006013G02.x3 1006 -

BE805023 | BE805023 | ss35d09.y1 *Gm-c1061 Glycine max*

BG582086 | BG582086 | EST483824 *GVN Medicago truncata*

AJ011609 | ATH011609 | *Arabidopsis thaliana*

10 BE023083 | BE023083 | sm90e08.y1 *Gm-c1015 Glycine max*

Furthermore, the invention relates to modifying NDR-NHL-genes or gene products. All proteins belonging to this family contain one (and sometimes even more than one) transmembrane 15 domain. *Arabidopsis* contains a large number of NDR-NHL genes, such as:

aad21459, aaf18257, aac36175, k10d20 (position 40852-41619),
aad21460, cab78082, aad21461, aad42003, aaf02134, aaf187656,
aad02133, cab43430, cab88990, cab80950, aad25632, aaf23842, al163812,
20 f20d21-35, t13m11-12, f1e22-7, t23g18, f5d14-4266, t32f12-16, f11f19-
11, f11f19-12, f11f19-13, t20p8-13, f12k2, f23h14, k10d20-44043,
k10d20-12, t19f11-6, t19f11-5, t10d17-10, f22o6-150, f3d13-5, m3e9-
80, t25p22-30, mhf15-4, mhf15-5, mrn17-4, mlf18-9, mgn6-11994, mjj3-
9667, f14f18-60, At1g17620 F11A6, At5g11890, At2g27080, At5g36970,
25 mlf18, At1g65690 F1E22, At4g01110 F2N1, At2g35980 f11f19,
At4g01410 F3D13, At1g54540 F20D21, At2g46300 t3f17, At5g21130,
At3g11650 T19F11, At5g06320 MHE15, At5g06330 MHF15, At2g01080
f15b18, At2g35460 t32f12, At2g27260 f12k2, At2g35970 f11f19,
At5g53730 MGN6, At5g22870 MRN17, At4g09590, At3g54200, At1g08160
30 T6D22, At5g22200, At3g52470, At2g35960 f11f19, At3g52460,
At5g56050 MDA7, At3g20590 K10D20, At1g61760 T13M11, At3g20600
K10D20, At1g13050 F3F19, At3g11660 T19F11, At3g44220, At1g64450
F1N19, At3g26350 F20C19 C, At4g05220, At5g45320 K9E15,
At4g23930, At4g13270, At4g39740, At1g45688 F2G19 W, At5g42860
35 MBD2, At1g32270 F27G20, At4g30660, At2g45430 f4123, At4g30650,
At1g69500 F10D13

and

40 ndr1, At2g27080; T20P8.13, At5g21130, At1g65690, At5g36970,

At1g54540, At5g06320, At5g11890, At1g17620, At3g11650, At2g22180,
At5g22870, At2g35980, At2g46300, At4g05220, At2g35460, At2g27260,
At4g01410, At5g22200, At1g61760, At3g52470, At5g53730, At4g01110,
At2g35960, At3g52460, At4g09590, At2g35970, At3g26350, At3g11660,
5 At3g44220, At1g08160, At2g01080, At5g06330, At5g56050, At3g20600,
NDR1, At3g54200, At3g20590, At4g39740, At1g32270 syntaxin, putative,
At1g13050, At5g45320, At3g20610, At4g26490, At5g42860, At1g45688,
At4g26820

10 NDR-NHL genes belong to a large family of which one of the
first identified is the defence-associated gene HIN1 (Harpin-
induced gene). HIN1 is transcriptionally induced by harpins
and bacteria, that elicit hypersensitive responses in tobacco.
It is thus believed that the genes of the invention also play
15 a role in the hypersensitive reaction. Especially (see also
chapter 8) since the genes of the invention bear relation to
brassinoid-like responses and since brassinoid pathway
compounds have been found to interact in this same defence
system in plants. Other plant species also contain members of
20 this large gene family, such as:

Plant homologs of the *Arabidopsis* NDR/NHL genes:

25 BG582276|BG582276|EST484016 GVN *Medicago truncata*
AV553539|AV553539|*Arabidopsis thaliana*
AC069325|AC069325|*Arabidopsis thaliana*
AV526693|AV526693|*Arabidopsis thaliana*
BG583456|BG583456|EST485208 GVN *Medicago truncata*
30 AW267833|AW267833|EST305961 DSIR *Medicago truncata*
BE997791|BE997791|EST429514 GVSN *Medicago truncata*
BG580928|BG580928|EST482657 GVN *Medicago truncata*
BF520916|BF520916|EST458389 DSIL *Medicago truncata*
AV544651|AV544651|*Arabidopsis thaliana*
35 AV543762|AV543762|*Arabidopsis thaliana*
AW559665|AW559665|EST314777 DSIR *Medicago truncata*
BG581012|BG581012|EST482741 GVN *Medicago truncata*
AV552164|AV552164|*Arabidopsis thaliana*
BE999881|BE999881|EST431604 GVSN *Medicago truncata*
40 AW031098|AW031098|EST274405 tomato

AI998763 | AI998763 | 701546833 *A. thaliana*
AW219286 | AW219286 | EST301768 *tomato*
BE124562 | BE124562 | EST393597 *GVN Medicago truncata*
AV540371 | AV540371 | *Arabidopsis thaliana*
5 AV539549 | AV539549 | *Arabidopsis thaliana*
BG647432 | BG647432 | EST509051 *HOGA Medicago truncata*
BE434210 | BE434210 | EST405288 *tomato*
BG725849 | BG725849 | sae42g02.y1 *Gm-c1051 Glycine max*
AP003247 | AP003247 | *Oryza sativa*
10 BE348073 | BE348073 | sp11all.y1 *Gm-c1042 Glycine max*
AW508383 | AW508383 | si40c06.y1 *Gm-r1030 Glycine max*
AI856504 | AI856504 | sb40b07.y1 *Gm-c1014 Glycine max*
BE556317 | BE556317 | sq01b07.y1 *Gm-c1045 Glycine max*
AA713120 | AA713120 | 32681 *Arabidopsis*
15 AV541531 | AV541531 | *Arabidopsis thaliana*
AI894456 | AI894456 | EST263911 *tomato*
AW704493 | AW704493 | sk53g11.y1 *Gm-c1019 Glycine max*
AW219298 | AW219298 | EST301780 *tomato*
BF425685 | BF425685 | ss03c11.y1 *Gm-c1047 Glycine max*
20 AV422557 | AV422557 | *Lotus japonicus*
BE190816 | BE190816 | sn79a08.y1 *Gm-c1038 Glycine max*
BG580331 | BG580331 | EST482056 *GVN Medicago truncata*
AV423251 | AV423251 | *Lotus japonicus*
AI896088 | AI896088 | EST265531 *tomato*
25 AV413427 | AV413427 | *Lotus japonicus*
AV426656 | AV426656 | *Lotus japonicus*
AV416256 | AV416256 | *Lotus japonicus*
AL385732 | CNS0690I | *Medicago truncatula*
AB016877 | AB016877 | *Arabidopsis thaliana*
30 AV419449 | AV419449 | *Lotus japonicus*
AI486269 | AI486269 | EST244590 *tomato*
AV411690 | AV411690 | *Lotus japonicus*
AV419925 | AV419925 | *Lotus japonicus*
AV418222 | AV418222 | *Lotus japonicus*
35 AV409427 | AV409427 | *Lotus japonicus*
AC005287 | AC005287 | *Arabidopsis thaliana*
AV426716 | AV426716 | *Lotus japonicus*
AV411791 | AV411791 | *Lotus japonicus*
BG351730 | BG351730 | 131E12 *Mature tuber*
40 BG046452 | BG046452 | saa54b12.y1 *Gm-c1060 Glycine max*
AI781777 | AI781777 | EST262656 *tomato*

BE451428 | BE451428 | EST402316 tomato
AI772944 | AI772944 | EST254044 tomato
AI895510 | AI895510 | EST264953 tomato
AW030762 | AW030762 | EST274017 tomato
5 AW218859 | AW218859 | EST301341 tomato
 BE203936 | BE203936 | EST396612 KVO *Medicago truncata*
 AV410289 | AV410289 | *Lotus japonicus*
 AW032019 | AW032019 | EST275473 tomato
 AW030868 | AW030868 | EST274158 tomato
10 AV421824 | AV421824 | *Lotus japonicus*
 BG646408 | BG646408 | EST508027 HOGA *Medicago truncata*
 AF325013 | AF325013 | *Arabidopsis thaliana*
 AC007234 | AC007234 | *Arabidopsis thaliana*
 AW217237 | AW217237 | EST295951 tomato
15 AC034257 | AC034257 | *Arabidopsis thaliana*
 AW625608 | AW625608 | EST319515 tomato
 AW031064 | AW031064 | EST274371 tomato
 AF370332 | AF370332 | *Arabidopsis thaliana*
 AB006700 | AB006700 | *Arabidopsis thaliana*
20 AW035467 | AW035467 | EST281205 tomato
 AL163812 | ATF14F18 | *Arabidopsis thaliana*
 AI896652 | AI896652 | EST266095 tomato
 AI730803 | AI730803 | BNLGH17970 Cotton
 AW034775 | AW034775 | EST278811 tomato
25

The invention provides the insight that RKS proteins or functional equivalents thereof play part in a signaling complex (herein also called the RKS signaling complex)
30 comprising molecules of RKS proteins, ELS (Extracellular Like SERK) proteins, NDR/NHL proteins and SBP/SPL (Squamosa Binding Protein) proteins, and the corresponding protein ligands (see for example table 3) whereby each of these proteins interplay or act in such a way that modifying genes, or modifying expression of genes, encoding ELS, RKS, NDR/NHL or SBP/SPL, 35 proteins or said ligands may lead to functionally equivalent results (Figure 5. Two-hybrid interaction experiments have for example shown in vitro interaction between RKS 0 and NDR0/NHL28 and members of the SBP/SPL family. Here we show
40 that in vivo the individual components of this signaling

complex are regulating identical processes, as based on functional genomics on transgenic plants, overexpressing or co-suppressing single components or combinations of components in this transmembrane signalling complex. ELS gene products 5 are derived from at least two different genes in the *Arabidopsis* genome. They show high homology on protein level with the corresponding transmembrane RKS gene products.

However, they lack a transmembrane domain while they do contain a signalling sequence at the N-terminal end. Therefore 10 these proteins are thought to be positioned within vesicles within the plant cell or at the outside of the plasma membrane, within the cell wall of the plant cell. A number of homologues have been detected in other plant species (see list 15 on page 3). ELS proteins are involved in the heterodimerizing complex with the RKS transmembrane receptor at the outer membrane site. ELS molecules are either in competition or collaboration with RKS molecules involved in the high affinity binding of the ligand. The signal transmitted from the ligand onto the RKS proteins is then transported over the membrane 20 towards the N-terminal site of RKS protein, located on the other site of the membrane. The activation stage of the RKS molecule is changed, as a result of transphosphorylation by dimerizing receptor kinase dimerizing partners. Subsequently 25 the signal is transmitted to other proteins, one family of such proteins is defined as the SBP/SPL family of transcription factors, the other family of proteins is represented by the NDR/NHL members.

The different obvious phenotypes created by modifying the 30 RKS gene products could be effected by one process regulating all different effects in transgenic plants.

All the phenotypes observed can be effected by the process of brassinosteroid perception. In chapter 1, RKS genes 35 are clearly involved in plant size and organ size. Loss of RKS expression results in a dwarf phenotype, similar as observed with brassinosteroid synthesis mutants. It was already known in literature that the phenotypes observed from modifying the

RKS genes are also observed when modifying the brassinosteroid pathway genes and/or their regulation, thereby altering the amount and nature of the brassinosteroids in plants.

Literature which describes the phenotypic effects of modifying

5 teh brassionosteroid pathway can, amogst others, be found in:

Plant Journal 26: 573-582 2001; Plant Journal 1996 9(5) 701-

713, genetic evidence for an essential role of

brassinosteroids in plant development; J. Cell Biochem Suppl.

21a 479 (1995) ; Mandava 1988 Plant growth-promoting

10 brassinosteroids, Ann. Rev. Plant. Physiol. Plant Mol. Biol.

39 23-52; Plant Physiol 1994 104: 505-513; Cell 85 (1996) 171-

182; Clouse et al. 1993 J. Plant Growth Regul. 12 61-66;

Clouse and Sasse (1998) Annu. Rev. Plant Physiol. Plant Mol.

Biol 49 427-451; Sasse, Steroidal Plant Hormones. Springer-

15 Verlag Tokyo pp 137-161 (1999).

It is thus believed, without being bound to any theory, that modification of the RKS genes will result in a modification of the brassinosteroid pathway, thereby giving the various phenotypes that are shown below.

20

"Functionally equivalent" as used herein is not only used to identify the functional equivalence of otherwise not so homologous genes encoding ELS, RKS, NDR/NHL or SBP/SPL proteins, but also means an equivalent gene or gene product of genes encoding ELS, RKS, NDR/NHL or SBP/SPL proteins in *Arabidopsis Thaliana*, e.g. identifying a homologue found in nature in other plants or a homologue comprising a deliberate nucleic acid modification, such as a deletion, truncation, insertion, or deliberate codon substitution which may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, and/or the amphipathic nature of the residues as long as the biological activity of the polypeptide is retained. Homology is generally over at least 50% of the full-length of the relevant sequence shown herein. As is well-understood, homology at the amino acid level is generally in terms of amino acid similarity or identity. Similarity allows for "conservative variation", i. e. substitution of one

hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine. Deliberate amino

5 acid substitution may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, and/or the amphipathic nature of the residues as long as the biological activity of the polypeptide is retained. In a preferred embodiment, all percentage homologies referred to herein refer
10 to percentage sequence identity, e.g. percent (%) amino acid sequence identity with respect to a particular reference sequence can be the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference sequence, after aligning the
15 sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, without considering any conservative substitutions as part of the sequence identity. Amino acid similarity or identity can be determined by genetic programs known in the art.

20 'Plant cell', as used herein, amongst others comprises seeds, suspension cultures, embryos, meristematic regions, callous tissues, protoplasts, leaves, roots, shoots, bulbs, gametophytes, sporophytes, pollen and microspores. A target plant to be modified according to the invention may be selected
25 from any monocotyledonous or dicotyledonous plant species, such as for example ornamental plants, vegetables, arable crops etc. 'Dicotyledoneae' form one of the two divisions of the flowering plants or angiospermae in which the embryo has two or more free or fused cotyledons. 'Monocotyledoneae' form one of the two
30 divisions of the flowering plants or angiospermae in which the embryo has one cotyledon. 'Angiospermae' or flowering plants are seed plants characterized by flowers as specialized organs of plant reproduction and by carpels covering the ovaries. Also included are gymnospermae. Gymnospermae are seed plants
35 characterized by strobili as specialized organs for plant reproduction and by naked sporophylls bearing the male or female reproductive organs, for example woody plants. 'Ornamental'

plants are plants that are primarily in cultivation for their habitus, special shape, (flower, foliage or otherwise) colour or other characteristics which contribute to human well being indoor as cut flowers or pot plants or outdoors in the man made 5 landscape, for example bulbous plant species like *Tulipa*, *Freesia*, *Narcissus*, *Hyacinthus* etc. 'Vegetables' are plants that are purposely selected or bred for human consumption of foliage, tubers, stems, fruits, flowers or parts of them and that may need an intensive cultivation regime. 'Arable crops' are 10 generally purposely bred or selected for human objectivity's (ranging from direct or indirect consumption, feed or industrial applications such as fibers) for example soybean, sunflower, corn, peanut, maize, wheat, cotton, safflower and rapeseed.

The invention provides a method for modulating a developmental 15 pathway of a plant comprising modifying a gene encoding for a gene product or protein belonging to a developmental cascade or signaling complex comprising modifying at least one gene encoding a gene product belonging to the complex of RKS proteins, ELS proteins, NDR/NHL proteins, SBP/SPL proteins and 20 ligand proteins. In one embodiment, the invention provides a method for modulating or modifying organ size. Plant or plant organ size is determined by both cell elongation and cell division rate. Modifying either one or both processes results in a change in final organ size. Increasing the level of 25 specific members of the family of RKS genes results in an increase in organ size, growth rate and yield. Modulating plant growth, organ size and yield of plant organs is the most important process to be optimized in plant performance. Here we show that modulating the level of members of the family of 30 the RKS signaling complex with a method according to the invention is sufficient to modulate these processes. The invention provides herewith a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein 35 said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating

cellular division during plant growth or organ formation, in particular wherein said gene comprises an RKS4 or RKS10 gene or functional equivalent thereof. Inactivation of endogenous RKS gene product results in a decrease in plant growth,

5 proving that the normal function of these endogenous RKS gene products is the regulation of growth and organ size. Use of a method according to invention for elevation of the levels of the regulating of the RKS signaling complex in plant cells is provided in order to increase for example the size of plant
10 organs, the growth rate, the yield of harvested crop, the yield of total plant material or the total plant size.

Decreasing the levels of endogenous RKS gene product is provided in order to decrease the size of plant organs, the growth rate, or the total plant size.

15 In another embodiment, the invention relates to cell division.

The mitotic cell cycle in eukaryotes determines the total number of cells within the organism and the number of cells within individual organs. The links between cell

proliferation, cell differentiation and cell-cycle machinery
20 are of primary importance for eukaryotes, and regulation of these processes allows modifications during every single stage of development. Here we show that modulating the level of members of the family of the RKS signaling complex is sufficient to modulate these processes. The invention provides

25 herewith a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and

30 RKS/ELS ligand protein allowing modulating cellular division during plant growth or organ formation, in particular wherein said gene comprises an RKS4 or RKS10 gene or functional equivalent Herewith the invention provides a method for modulating the number of cells to be formed within an

35 eukaryotic organism as a whole or for modulating the cell number within individual organs is, which of primary importance in modulating plant developmental processes,

especially of arable plants. Here we show that members of the RKS signaling complex are able to regulate the number of cellular divisions, thereby regulating the total number of cells within the organism or different organs.

5

In a further embodiment, the invention relates to the regeneration of apical meristem. Modification the levels of different RKS and ELS genes within plants allows the initiation and / or outgrowth of apical meristems, resulting 10 in the formation of large numbers of plantlets from a single source. A number of gene products that is able to increase the regeneration potential of plants is known already. Examples of these are KNAT1, cycD3, CUC2 and IPT. Here we show that modulation of the endogenous levels of RKS genes results in 15 the formation of new shoots and plantlets in different plant species like *Nicotiana tabacum* and *Arabidopsis thaliana*.

Herewith the invention provides a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein 20 said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein, allowing modulating apical meristem formation, in particular wherein said gene comprises an ELS1, RKS0, RKS3, RKS4, RKS8 or RKS10 25 gene or functional equivalent thereof. A direct application of such a method according to the invention is the stable or transient expression of RKS and ELS genes or gene products in order to initiate vegetative reproduction. Regeneration can be induced after overexpression of for example RKS0 and ELS1; or 30 by co-suppression of for example the endogenous RKS3, RKS4, RKS8 or RKS10 genes. Overexpression or co-suppression of these RKS and ELS gene products can be either transient, or stable by integration of the corresponding expression cassettes in the plant genome. A further example of essentially identical 35 functions for for example ELS1 and RKS0 overexpressing plants is for example shown in the detailed description, example 3, where both transgenic constructs are able to induce the

regeneration capacity of in vitro cultured *Arabidopsis* callus. Another example comprises functional interaction between RKS and SBP proteins which was shown by studies in transgenic tobacco plants in which SBP5 and RKS0 were both overexpressed under the control of an enhanced 35S promoter. At the tip of double overexpressing plants, embryostructures appeared whereas in the SBP5 overexpressing plants alone or the RKS0 overexpressing plants alone no phenotype was detectable at the root tips of transgenic tobacco plants. These results show that both RKS and SBP proteins are involved together in a signaling cascade, resulting in the reprogramming of developmental fate of a determined meristem. Furthermore, it is herein also shown that several RKS genes are able to regulate proper identity and development of meristems and primordia. The invention for example also relates to fasciation. Fasciation is normally a result from an increased size of the apical meristem in apical plant organs. Modulation of the number of cells within the proliferating zone of the shoot apical meristem results in an excess number of cellular divisions, giving rise to excess numbers of primordia formed or to stems in which the number of cells is increased. The invention herewith provides a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating fasciation, in particular wherein said gene comprises an RKS0, RKS3, RKS8 or RKS10 gene or functional equivalent thereof. Here we for example show that modulation of the levels of RKS gene products in plants like *Arabidopsis thaliana* can result in fasciated stems. A direct application as provided herein is the regulated formation of fasciation in plant species in which such a trait is desired like ornamental plants. Regulation of the initiation and extent of fasciation, either by placing the responsible RKS encoding DNA sequences under the control of stage or tissue specific

promoters, constitutive promoters or inducible promoters results in plants with localized or consitutive fasciation of stem tissue. Another application is modulating the number of primordia by regulation of the process of fasciation. An 5 example is provided by for example sprouts, in which an increased number of primordia will result in an increased numbers of sprouts to be harvested. Fasciation can also result in a strong modification in the structural architecture of the inflorescence, resulting in a terminal group of flowers 10 resembling the *Umbelliferae* type.

Identical phenotypes can be observed when transgenic plants are produced that contain the NHL10 cDNA under control of an enhanced 35S promoter. The resulting phenotype of the resulting flowers show that flower organ primordia are 15 switched in identity, similar as observed for RKS10 and RKS13. These meristematic identity switches are normally never observed in *Arabidopsis* and the fact that two different classes of genes are able to display the same phenotypes in transgenic plants is a clear indication for a process in which 20 both members of the RKS and the NDR/NHL families are involved. The invention also relates to root development. Fasciation is normally a result from an increased size of the apical meristem in apical plant organs. Modulation of the number of cells within the proliferating zone of the root apical 25 meristem results in an excess number of cellular divisions, giving rise to excess numbers of primordia formed or to roots in which the number of cells is increased. Adaptation to soil conditions is possible by regulation of root development of plants. Here we describe several processes in root development 30 that can be manipulated by modification of the levels of RKS signaling complex within the root. The invention provides a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein 35 belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating root development, in particular

wherein said gene comprises an ELS1, ELS2, RKS1, RKS3, RKS4, RKS6, RKS8 or RKS10 gene or functional equivalent thereof. Root length, a result by either root cells proliferation or elongation, can for example be increased by overexpression of for example RKS3, RKS4, RKS6 and ELS2, or inactivation of the endogenous RKS10 gene product. Root length can also be decreased by decreasing of endogenous RKS1 levels or by strong overexpression of RKS10. The initiation of lateral roots is also regulated by RKS gene products. Overexpression of for example RKS10 can result in a strong increase in the initiation and outgrowth of lateral roots. Co-suppression of RKS1 also resulted in the initiation and outgrowth of large numbers of lateral roots. Root hair formation and elongation is important in determining the total contact surface between plant and soil. A strong increase of root hair length (elongation) can be obtained by overexpression of ELS1 and RKS3 gene products. As the roots of terrestrial plants are involved in the acquisition of water and nutrients, anchorage of the plant, synthesis of plant hormones, interaction with the rhizosphere and storage functions, increasing or decreasing root length, for example for flexible adaptations to different water levels, can be manipulated by overexpressing or cosuppressing RKS and / or ELS gene products. Modulation of the total contact surface between plant cells and the outside environment can be manipulated by regulation lateral root formation (increased by RKS10 overexpression and co-suppression of RKS1). Finally the contact surface between plant cells and the soil can be influenced by modulation of the number of root hairs formed or the elongation of the root hairs, as mediated by ELS1 and RKS3.

In a further embodiment, the invention relates to apical meristem identity. All parts of the plant above the ground are generally the result on one apical shoot meristem that has been initiated early at embryogenesis and that gives rise to all apical organs. This development of a single meristem into complex tissue and repeated patterns is the result of tissue

and stage-dependent differentiation processes within the meristems and its resulting offspring cells. The control of meristem formation, meristem identity and meristem differentiation is therefore an important tool in regulating plant architecture and development. Here we present evidence the function of RKS and ELS gene products in regulation of the meristem identity and the formation and outgrowth of new apical meristems. The invention provides a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating meristem identity, in particular wherein said gene comprises an ELS1, RKS8, RKS10 or RKS13 gene or functional equivalent thereof. Introduction of for example the RKS10 gene product or an other member of the RKS signaling complex under the control of a tissue and / or stage specific promoter as provided herein allows localized and time regulated increases in the levels of gene product. For example the meristematic identity in a determined meristem might thereby be switched back into an undetermined meristem, thereby changing for example a terminal flower into an undetermined generative meristem.

Another application might be found in changing the meristematic identity at an early time point, during early vegetative growth, thereby switching the vegetative meristem into a generative meristem, allowing early flowering. Modulation of meristem identity in terminal primordia, like for example as shown in Figure 30, where flower organ primordia are converted into terminal flower primordia, allows the formation of completely new types of flowers and fused fruitstructures. Constitutive overexpression of RKS gene products results in plants with many apical meristems, as can clearly been seen in Figure 29, where RKS10 overexpression results in an extremely bushy phenotype.

In another embodiment, the invention relates to male sterility. Male sterility is a highly desired trait in many plant species. For example, manipulation of pollen development is crucial for F1 hybrid seed production, to reduce labour

5 costs and for the production of low-environmental impact genetically engineered crops. In order to produce hybrid seed from inbred plant lines, the male organs are removed from each flower, and pollen from another parent is applied manually to produce the hybrid seed. This labour-intensive method is used
10 with a number of vegetables (e.g. hybrid tomatoes) and with many ornamental plants. Transgenic approaches, in which one or more

introduced gene products interfere with normal pollen initiation and development is therefore highly desired.

15 Especially when the number of revertants (growing normal pollen) is extremely low.

Male sterility in plants is a desired trait that has been shown already in many plant species as a result of the inactivation of expression of a number of genes essential for
20 proper stamen development, mitotic divisions in the pollen stem cells, or male gametogenesis. A method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein, allowing modulating pollen development, in particular wherein said gene comprises an ELS2 or RKS10 gene or functional equivalent thereof.

25 30 35 Here we present data that show that overexpression of gene products, like transmembrane receptor kinases (RKS) and extracellular proteins (ELS) can also result in the formation of male sterility. The ability to induce male sterility by overexpressing specific genes as provided herein allows the opportunity to produce transgenic overexpressing plants in which the pollen development is inhibited. Stable single copy homozygous integration of such overexpressing traits into the

plant genome will render such plants completely sterile, making them excellent material for the production of F1 hybrid seed. Furthermore, the combined integration of a male sterility inducing overexpressing gene coupled directly with

5 another desired transgene result in transgenic plants which are unable to produce transgenic seed, making these transgenic plants excellent material for outside growth without problems affecting transgenic pollen spreading throughout the environment, thereby eliminating possible crosses

10 with wild plant species or other non-transgenic crops. The combination of a desired transgene flanked on both sites by different male-sterility inducing overexpressing genes would decrease the frequency of pollen formation to an extremely low level. An example is an overexpressing construct of RKS10

15 at the 5'end of integrated DNA fragment, the desired transgene expression cassette in the middle and at the 3'end of the integrated DNA the ELS2 overexpressing construct. This complete DNA fragment is integrated into the genome by conventional techniques, like particle bombardment,

20 *Agrobacterium* transformation etc. Another possible application concerns the modification of pollen in ornamental plant species like lily, where the release of pollen from cut flowers can be avoided by making transgenic plants in which pollen development is initiated by release from the stamen is

25 prevented (a desired trait that can be obtained by overexpressing for example ELS2, resulting in partial pollen development). Hereby the ornamental value of the stamen with pollen is not lost, but release of pollen is inhibited.

Furthermore, surprisingly we observe that NDR NHL gene

30 products share homology with the family of syntaxins, involved in vesicle transport, positioning of cell wall formation and cytokinesis.

Table 1

Homology between members of the syntaxin family and the NDR NHL family

5 NHL10= At2g35980

maaeqplnga fygpsvppa pkgyyrrghg rgcgccllsl fvkviisliv ilgvaalifw
livrpraikf hvtdasltrf dhtspdnlr ynlaltpvpr npnkriglyy drieahayye
gkrfstitlt pfyqghkntt vltptfqgqn lvifnagqsr tlinaerisgv ynieikfrlr
vrfklgdlkf rrikpkvdcd dlrlplstsn gttttstvfp ikcdfdf

10

At1g32270 syntaxin,

MVRSNDVKFQ VYDAELTHFD LESNNNLQYS LSLNLSIRNS KSSIGIHYDR FEATVYYMNO
RLGAVPMPLF YLGSKNTMLL RALFEGQTLV LLKGNERKKF EDDQKTGVYR IDVKLSINFR
VMVLHLVTWP MKPVVRCHLK IPLALGSSNS TGGHKKMLLI GQLVKDTSAN LREASSETDHR
15 RDVAQSKKIA DAKLAKDFEA ALKEFQKAQH ITVERETSYI PFDPKGSFSS SEVDIGYDRS
QEQRVILMESR RQEIVLLDNE ISLNEARIEA REQGIQEVKH QISEVMEMFK DLAVMVDHQG
TIDDIDEKID NLRSAAAQGK SHLVKASNTQ GSNSSSLFSC SLLLFFFLSG DLCRCVCVGS
ENPRLNPTRR KAWCEEDEE QRKKQQKKKT MSEKRRREEK KVNKPNGFVF CVLGHK*

20

Below the homology is shown between NHL10 (Upper line) and a syntaxin protein. (bottom line). The identical amino acids are shown in the middle line.

25

IVRPRAIKFHVTDASLTRFDHTSPDNILRYNLALTVPVRNPNKIRIGLYYDRIEAHAYYEG
VR KF V DA LT FD S N L Y L L RN IG YDR EA YY
MVRSNDVKFQVYDAELTHFDLESNNN-LQYSLSLNLSIRNSKSSIGIHYDRFEATVYYMN

30

KRFSTITLTPFYQGHKNTTVLTPTFQGQNLVIFNAGQSRTLNAERISGVYNIEIKFRLRV
R FY G KNT L F GQ LV GVY I K
QRLGAVPMPLFYLGSKNTMLLRALFEGQTLVLLKGNERKKFEDDQKTGVYRIDVKLSINF

35 RFKLGDLKFRRRIKPKVDCDDLRLPLSTSNGTTT

R L KP V C L PL T
RVMVLHLVTWPMKPVVRCH-LKIPLALGSSNST

That syntaxins and NDR/HNL genes share large homology becomes even more clear when performing a database search using the following site:

http://mips.gsf.de/proj/thal/db/search/search_frame.html

5 searching for homologous sequences with the sequence At1g32270

gene code:

predicted function:

10	At1g32270 syntaxin, putative	Syntaxin
	At5g46860 syntaxin related protein	Syntaxin
	AtVam3p (gb AAC49823.1)	
	At4g17730 syntaxin	Syntaxin
	At5g16830 syntaxin homologue	Syntaxin
	At3g11650 unknown protein	Putative syntaxin
15	At2g35460 similar to harpin-induced protein	Putative syntaxin
	At5g06320 harpin-induced protein-like	Putative syntaxin
	At2g35980 similar to harpin-induced protein	Putative syntaxin
	At1g65690 hypothetical protein	NDR HNL
	At4g05220 putative protein	Putative syntaxin
20	At3g05710 putative syntaxin protein	Syntaxin
	AtSNAP33	
	At2g27080 unknown protein	NDR HNL
	At3g52470 putative protein	Putative syntaxin
	At1g61760 hypothetical protein	Putative syntaxin
25	At5g21130 putative protein	NDR HNL
	At3g52400 syntaxin-like protein synt4	Syntaxin
	At2g35960 putative harpin-induced protein	Putative syntaxin
	At5g06330 harpin-induced protein-like	Putative syntaxin
	At5g26980 tSNARE	Syntaxin
30	At5g36970 putative protein	Putative syntaxin
	At3g44220 putative protein	Putative syntaxin
	At3g03800 s-syntaxin-like protein	Syntaxin
	At2g35970 putative harpin-induced protein	Putative syntaxin
	At4g09590 putative protein	Putative syntaxin
35	At4g23930 putative protein	
	At1g61290 similar to syntaxin-related protein	Syntaxin
	At3g11660 unknown protein	Putative syntaxin
	At1g54540 hypothetical protein	Putative syntaxin
	At3g24350 syntaxin-like protein	Syntaxin
40	At5g22200 NDR1/HIN1-like	NDR HNL

	At1g11250 syntaxin-related protein At-SYR1	Syntaxin
	At5g53880	
	At3g11820 putative syntaxin	Syntaxin
	At3g54200	Putative syntaxin
5	At5g05760 t-SNARE SED5	Syntaxin
	At5g53730	Putative syntaxin
	At4g03330 SYR1-like syntaxin 1	Syntaxin
	At3g47910	
	At5g08080 syntaxin-like protein	Syntaxin
10	At5g11890	Putative syntaxin
	At1g17620	Putative syntaxin
	At2g22180	Putative syntaxin
	At5g22870	Putative syntaxin
	At2g46300	Putative syntaxin
15	At2g27260	Putative syntaxin
	At4g01410	Putative syntaxin
	At5g22200	Putative syntaxin
	At4g01110	Putative syntaxin
	At3g52460	Putative syntaxin
20	At3g26350	Putative syntaxin
	At1g08160	Putative syntaxin
	At2g01080	Putative syntaxin
	At5g56050	Putative syntaxin
	At3g20600	Putative syntaxin
25	At3g20590	Putative syntaxin
	At4g39740	Putative syntaxin
	At1g32270	Putative syntaxin
	At1g13050	Putative syntaxin
	At5g45320	Putative syntaxin
30	At3g20610	Putative syntaxin
	At4g26490	Putative syntaxin
	At5942860	Putative syntaxin
	At1g45688	Putative syntaxin
	At4g26820	Putative syntaxin

35

40 This observation provides the explanation for understanding the mechanism by which the RKS / NDR-NHL complex functions. Cell wall immobilized RKS gene products (containing the

extensin-like extracellular domain) respond to a local ligand signal, in combination with the heterodimerizing ELS protein(s) either as homodimers, as RKS heterodimers or in combination with the heterodimerizing ELS protein(s).

5 Predicted ligands for the RKS / ELS receptor binding consist of peptide ligands (based on the IRR ligand binding domain of this class of receptors). These ligands are normally produced as a pre pro protein. The N-terminal signal sequence is removed by the transport through the Golgi system and
10 allows modification of the ligand at this stage (e.g. glycosylation). The ligands can then be secreted after which further processing is possible (e.g. proteolytic cleavage, removal of sugar groups etc.) The resulting peptide, possible as a monomer or a (hetero)dimerizing molecule binds the
15 transmembrane receptor complex with high affinity, resulting in transmission of the signal from the ligand through the transmembrane receptor component towards the other site of the membrane.

One class of ligands interacting with the RKS and / or ELS
20 receptors consists of the family of pre(pro)proteins shown hereunder in table 3.

Table 3 Ligands within the RKS signaling complex (herein also called RKS/ELS ligand proteins)

5 For each ligand (A to N) the genomic structure before splicing and processing 5'- towards 3' is given. Exons are indicated in large letters; introns and surrounding sequences (including leader 5'-and trailer sequences 3'-) are indicated in small letters.

10 Beneath each DNA sequence the amino acid sequence of the pre-pro-peptide is given. The first line represents the signal sequence

10 The second (set of) lines represents the pro-peptide.
The last line represents the conserved Cysteine motif.

A. At1g22690

15 1 attaaacgcc aaacactaca tctgtgtttt cgaacaatat tgcgtctgcg tttccttcat
61 ctagtctct caggtgcaca atgtctgaac taagagacag ctgtaaacta tcattaagac
121 ataaaactacc aaagatataa gctaataatgaa aaattactct catttccacg taacaaatttg
181 agtttagctta agatatttagt gaaaacttagt ttgaattttc ttctttttct tcacatgcac
241 ctccgaaaaa agggaaaccaa tcaaaaactgt ttgcataatca aactccaaaca ctttacagca
301 aatgcataatcttataatctgtg attatccaa taaaactctg tgattttatgt ttggctccag
361 ctagtgcataatctgtt gatctctataa caacatgatg aatttttcag aaaataaaaaa
421 gtagctgaaa tggatctataa taaagaatca tccacaagta ctattttcac aactacttc
481 aaaatcaacta ctcaagaaat ATGAAGAAGA TGAATGTGGT GGCTTTGTT ACGCTGATCA
541 TCTCTTTCT TCTGCTTCT CAGGtaaact gttaaaaacca ttttcaagac tacccctttt
601 ctatttcaga caaaccaaaaag taaaacaatg aaaaatctct ctggcttttc atagGTACTT
661 GCAGAGTTGT CATCATCCAG CAACAAATGAA ACTTCCTCTG TTTCTCAGgt aagagtgtata
721 caaaaacata ctaaacaacac tttcaagaga gtaatataa agggaaatgtt ggcttctttt
781 ttttgtgtgt aatcagACGA ATGACGAGAA CCAAACAGC GCGTTAAAGA GAAACATACCA
841 CCATCGTCCA AGAACATCAGtt agtctactct ttcaacactc taattccctt gttctaagta
901 ttttttttgc cccccacaaac cttttttta taaaatgagc caatttttat agATTGTGGG
961 CATGCATGCG CAAGGAGATG CAGTAAGACA TCGAGGAAGA AAGTTGTCA CAGAGCCTGT
1021 GGAAGTTGTT GTGCCAAGTG TCAGTGTGTG CCGCCGGGAA CCTCCGGCAA CACAGCATCA
1081 TGTCTTGCT ACGCCAGTAT CCGTACACAT GGCAATAAAC TCAAATGTCC TAAAGact
1141 tctcattttct caactataatgt ctcatcttct gattatgtt ctctttttgt tatgttgcat
1201 gtgtgtatgtg tgatgttattt attatgttga ttgttgacat aattcaacta tataatttgt
1261 atcgatccg aataaataaga tgatgtgattt tattggctat taatgtttttt tttttttttt
1321 ttgggcacaa tggatattaa gtttaaaca tctgatattta ttggatcaa aaaaacaacaa
1381 agtttcattt tcatattaaac acaaaatctc catacatatt accaaaccaa aaaaataacac
1441 aagggggaga gagaccaacg gttcttggtt cagagttgc atcttgtttg agccgtcacc
1501 gtttctttaga cttaacagcc acaacacctt tataaagctt cacgcgatcc ttcaacgcatt
1561 ctgcggcaggccgagccacc ttattgtttg gatcaaacaa caaaacttct tcaaacgcatt
1621 tcaatgcacaa aggcc

45 MKKMNVVAFVTLIISFLLSQVLA

ELSSSSNNETSSVSQTNENQTAFKRTYHHRPRIN

CGHACARRCSKTSRKVCHRACGSCCAKCQCVPPTSGNTASCPCYASIRTHGNKLKCP*

B. At1g74670

1 gaaaaaaaaaaga agaaaaagata atgggtccgta ttaatatagt tgaaaaacttg aaactacttt
 5 61 ttagtttcta tataatacag tagacttaggg atccagttga gtttctttct ttattttgag
 121 tttgtgttta tgtttgattt tacgtttttta tatttaataa agatattttta cgaattatgg
 181 ttttattttgg gtagaaagttg tagaatgact taaaacaatca agtggcagaa tgagatatat
 241 aaagtaataat aataatatgtt ccgttattaa cttattgtac atgtaatgaa ggaagcttac
 301 acacacacac cttctataaaa tagctgacaa aactgggtgt tacacacaac acattcataa
 361 atctctcaaa gtaagaacta agagctttac tacagtccta ctctctcac acatctctc
 421 tctctcaaga gcttagtcATG GCCAAACTCA TAACTTCTTT TCTCTTACTC ACAATTAT
 481 TCACCTTCTGT TTGTCCTCACT ATGTCAAAAG AAGCTGAGTA CCATCCAGAA AGTgttaagtt
 541 tttatttttt ggtaaaatag aaagtgttaag ttttataatt cattcaattt ttttgcctt
 601 tccctttcta tttattgtca taaaatctaatt acccgcgtt aaatttggttt tgaatttaaa
 661 cagTATGGAC CAGGAAGTCT GAAATCATAc Cgtaaatggaa aactttttct tcttttatga
 721 atcttggttc ttattttata tcaaataaaa actcgattat catgattgca gAATGTGGAG
 781 GACAATGCAC AAGGAGATGT AGCAACACAA AGTATCATAA GCCATGCATG TTCTTCTGCC
 841 AAAAGTGTG TGCTAAATGC CTTTGTGTCC CTCCAGGCAC GTACGGCAAC AAACAAGTGT
 901 GTCCTTGT TA CAACAACTGG AAGACTCAAC AAGGTGGACC AAAATGTCCA TAAacaaaaa
 961 cattgagaga gaaaccccaa tctgtttctt atttttttta atttttcca gtatgtttt
 1021 gttgtcgta tggtaaaattt atagtgtttt tgccggatc attttatcatc gataaaacaat
 1081 atcatataaa atcttctatg ttttttttcac gttttgtttc tttttgtta gtcatacac
 1141 gaaatgtgtta tggacccctt aatttaggaat atataaaaatt ttatttttta attagataat
 1201 ctttcgtata gttaaaattt caaggattac ttttggatcg tttgggacaa tctatttat
 1261 attttacttt ctaagtttgcataactatctt cttaaaatgg ttagacagag tcctaatgtat
 1321 ttttagtataat ttgttactat ttgttacgc ttcaaaaattt tggaaactttt ccaaagtgg
 1381 ctatataat ttgatttactt aatctgcgtt tccttcttagt tttttacaat tatggagatt
 1441 ttgcacgat gat

30

MAKLITSFLLTILFTFVCLTMS

KEAEYHPESYGPGLKSQ

35 CGGQCTRRCSNTKYHKPCMFFCQKCCAKCLCVPPGTYGNKQVCPCYNNWKTQQGGPKCP *
 CGGQCTRRCSNTKYHKPCMFFCQKCCAKCLCVPPGTYGNKQVCPCYNNWKTQQGGPKCP *

C. At1g75750

1 cacaactttt atacgcacca ccaaccgacc cattttgaaa aagagaaaaat aaaccacaaa
 5 61 aacacacata aataatatgc tgataacaat gtcttaaaaaa tctatttacc atttctagta
 121 atcaatatct attgcaaaaa atatttataa gaataacaat gaaaatgtat aaaaatacaaa
 181 tgatttctca attacctaaa aaatataaaa atgtcttact ttattttcag ccactgttgg
 241 aaagtacttg caatcatatc gtattttgaa ttataaaaact cagaaacaat tattttccct
 301 gaaaagttaa aacttttaat aagatattta taaaataaaa agaatagtct agaccgaaaa
 361 tgggttcgggt tgtcatcca aaggagtgtct ataaaatgaa ccctcagaat ttcatttagg
 421 acacaacaac taaaaccaca tttatcatta cagtcgtatt tgagctaat tcttcatca
 481 taaaactctcc ttggagaatc ATGGCTATTT CAAAAGCTCT TATCGCTTCT CTTCTCATAT
 541 CTCTTCTTGT TCTCCAATC GTCCAGGCTG ATGTCgtacg tcttttcat cacaactaa
 601 ttatactcaa tataatactt atgttttcaa aaacatattt ctcacatgtt acaacaatat
 661 tcttcgcagGA AAACTCACAG AAAGAAAAATG GTTACGCAAA GAAGATCGgt aatttatatga
 721 tttttatcaa acctaaccgtt aaatttagag tgagatataat aatctgtgtt ttttttttt
 781 gtatataatg ATTGTGGGAG TCCGTGTGTA GCACCGTGC GGCTTCGAG GAGGCCGAGG
 841 CTGTGTCACA GAGCGTGCAG GACTTGCTGC TACAGGTGCA ACTGTGTGCC TCCGGGTACG
 901 TACGGAAACT ACGACAAGTG CCAGTGCTAC GCTAGCCTCA CCACCCACGG TGGACGCCGC
 961 AAGTCCCCAT AAgaagaaaac aaagcttta attgctggg ataatgggac gatgtcggtt
 1021 tgtagtatt tacttggcg tataatatgtg gatcgaataa taaacgagaa cgtacgttgt
 1081 cgttgtgagt gtgagttactg tattatataat ggttctattt gttttactt gcaagtttc
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 1321 tgataactac tagactagct gagtataaga atattgtat atatatttgc ggacaatttt
 1381 gaatttattt taccatttt taatcagcgc catataaaaa taattcttgtt ttgcgttata
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30

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35

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D. At2g14900

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 5 61 tcgtttttt cattggagtt tgactctact aagtgtgtt tcctcgcgta gtaagaattg
 121 gttatggatt agaccgtatc gatctaaaga tgcataaaga aaaaaatgt ggttgtgtaa
 181 agtaaatatg tagattgtgg cggattaaag tatgttttga ttacatcatcat tattttatt
 241 ttttcatgaa ttctaaatgt aaagttcttta taatcttgc ttacttttta caaattgtaa
 301 ggattactct gaaatttgggt atcgaattct aagacaataa caaaataaca atgactgaac
 10 361 aagtgtataa aacataatgg aaggaataat actgcagttt tattaaataac taaagaagtt
 421 ggttagattgg cctataaaaag gagaataaaag agaccacaag aaggcttattt attcggggac
 481 taaaagaaajc caaaagaaaac ATGAAAATAA TAGTCTCCAT CTTAGTGTAA GCCTCTCTTC
 541 TTCTAATCAG TTCATCTCTT GCTTCGGCTA CTATATCAGG ttgggtctaa tctcttcaag
 601 aatcttcttc tctcttatttt ttttttcttc ataaagttt tagtattat attggtttag
 661 gtcacaattt tttctttatg cttctgtttc cataagaaaa atattacaaa tattaaactag
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 1141 CGTTAGAGAA ATGGTGTGGA CAGAAATGTG AAGGGAGATC CAAAGAAGCG GGGATGAAAG
 1201 ATCGGTGTTT GAAGTATTGT GGGATATGTT GCAAAGACTG TCAGTGTGTT CCTTCAGGCA
 1261 CTTATGGAA TAAGCATGAA TGTGCTTGCT ATCGTGACAA GCTCAGTAGC AAAGGCACTC
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 1441 ttcttattttt acgaattttgtt ttgggtcttt ttgaagttt tttttttttt ttgttttttcat
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 1621 aatttagatgtt gtcaccccgta taaaacaaat tgaatcgaat tttttttttt aatattttaa
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 1741 gagaagaacg tgggttggtaa aataatattt atgggttga gacaactttt aagagatttt
 1801 aaaaagactg actaacgtgt taggttcatc acgt
 35

MKIIIVSILVLASILLISSSLASATIS

DAFGSGAVAPAPQSKDGPALEKW

40 CGQKCEGRCKEAGMKDRCLKYCGICCKDCQCVPSGTYGNKHECACYRDKLSSKGTPKCP*
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E. At2g18420

1 gccaatgggt aactgaggaa gaaggataag accaaaaaaaaa aaactaaaaat ggacagattg
 5 61 aatttagtaaa aagataaaatt ctaaaaaccg aaacaaatctt taagttggtg tatatacacatc
 121 tgcattgacc aacaaaagaa attagactga aattttatgg aaaaatgatct tgtaaaggca
 181 tattatataat ttaattttagg aaatgaatgt taaaatccctt aaattgtttt gatttcacaa
 241 aaggataaaag aaatatttgt tacatacacatc ttaatgtgtt gacccaaaca aataaaaatgt
 301 gataagaaac aataaaaacca ttttgaccaa agttctata gttttaatat tctttaattg
 361 tcattttgtta gtgactaata atattacattt aaacctaattg tataaataga agccccatct
 421 tctacccgttataatttgc aacaacccaa aacattcattt tgctattttg tctccctttt
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 1741 tcccatcttc ttgttacaa attccccgtt gtttgcacaa tc

35 MAVFRVLLASLLISLLVLDVHAA

DMVTSNDAPKID

CNSRCQERCSLSSRPNLCHRACGTCCARCNCVAPGTSGNYDKCPCYGSLLTHGRRKCP*

40

F. At2g30810

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	61	attaaaataac	agcttatttt	ctcttatttc	ttagtgatta	aaatatttaa	aatacagacc
	121	aaaatttaatt	gtttatgtta	atataatttc	tccttaatcc	tttatattaa	aattgtataa
	181	tgcatgttagt	taataaaatttgc	ttttccaaaa	ttcattcata	atTTTATTC	taaatttattt
	241	ttgtcaagaa	aacacatattt	tgaataatttgc	aatgtttctt	tgttattgtat	aattttcttg
10	301	tatTTTAAAT	taccttctt	actatgccaa	tgttattgtat	tataatttagt	tttaacatgtt
	361	atccgtaaaa	atatacataag	aaaatccaaa	gtgaaaaataag	agatcaaaAT	GATGAAGCTC
	421	ATAGTTGTCT	TTGTTATATC	CAGTTGTTG	TTTGCTACTC	AATTTCTAA	Tgtaaaaatt
	481	attatttattt	tcttcatattt	atgattttatg	aattcagaga	aataaaagttt	tttttttttat
	541	gtgtgtatgt	acagGGTGAT	GAATTAGAGA	GTCAAGCTCA	AGCACCTGCA	ATCCATAAAGg
15	601	tatTTTAAAT	ttataaaata	tcaaaatactg	aataataat	aataaaatata	ttacaacaag
	661	aatatcaat	tttattttca	aactactata	ttttttttata	tttttattgtat	aacacaatgt
	721	tatTTTATTA	tcgtcttcatt	tgattttgcatt	tcttaattttgc	tttttttttat	ccaaccaattt
	781	tcaAAATGGA	GGAGAAAGGCT	CACTTAAACC	AGAAAGttaaa	ttgttttttttt	gatattttttt
	841	ttatTTTATAT	agtaaaatgtat	tgatcaaatttgc	acaactttttttt	taatTTTAAAT	gttggattttat
20	901	atTTTCTGA	agAAATGTCA	AAGGCATGTG	AATATCGATG	TTCCGGCGACA	TCTCACAGGA
	961	AACCATGTTT	GTTTTTTTG	AACAAATGTT	GTAACAAATG	TTTGTGTGTA	CCATCGGGAA
	1021	CATATGGACAA	CAAAGAAGAA	TGTCCTTGC	ACAATAATTG	GACGCCACAA	GAAGGTGGAC
	1081	CAAATGTC	ATGAAacaa	aaaattgtta	aacaaatata	aatTTTATCG	ttgtttatctc
	1141	tcaataaaat	ctatgtttgt	aatccttgc	tttcaatata	gaataataata	ttggaggTTTC
	1201	ataatTTCTT	cttattcaaaat	attaaatgtta	atgcacaaat	aaatttgaagg	gactttggacc
	1261	ttttcgtgt	agttttttct	ttaaatcagc	aacaatttttag	atTTTATATT	tcacttttac
	1321	aaacacaaaa	catggatgt	ctttaactct	catccaaaca	aatgcattt	ctctttttct
	1381	ttttctaaac	atttcacaac	aatataccat	attatatactt	agatataatgt	tctttttttaa
	1441	ttgtatTTAT	tttaggcccatt	ttttttaaatc	gtgtttttgtt	agattgaccc	atggaaatgtt
30	1501	gacatTTTTT	aacatttctt	aatatgacta	aaaatgttata	aagatattttt	ataatatatt
	1561	tgctcttattt	aaaatgattt	aataaaaaat	aata		

MMKLIIVVFVISSLLEFATQFSNG

35 DELE SQAQAPAIHKNGGEGSLKPEE

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G. At2g39540

1 taatgcata ctttaatct ataatatata ttagatgtga cttaaggaat ttcaatagtt
 61 atacataata ataaaaatga atatttgtta gtgttacaaa ctgtgtgtca taatcatcat
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 181 tgcactataa gaaataaaatt tacaatttaa aaaatgctc aatactggtt aaaaaaaaaa
 241 ctttcaatac tagtattata ctacttactt agtcaaaaaa gtttatgaat atggtttttt
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 541 TCCTCACATC TTCATTTCT GTACTTTCAA GTGCTGATTC GTgttaagtgt ttacttaatc
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 1081 taacacatca acgaatctttt aatctttatcatgtatata attatcagag caccctaaaca
 1141 ttgcgcgtt ttgtgattat acaaagtaac atcgtgctgt ttttgacttt tgaaaaccac
 1201 agatccaaaa actgtttact ttccctctaag agaaagccaa gccgagttag tccaaagcgag
 1261 ttttgagaga ttccgttact cactaccggaa gaacgacgt atgtcagaga cccgcgtgtc
 1321 aatcgattcg gaccgatcta agtccggagga agaagacgaa gaagagtatt ctccac

MKLVVVQFFIISLLLTSFSVLSSA

30

DSS

CGGKCNVRC SKAGQHEE CLKYCN ICCQKCN CVP SGT FGH KDEC PCY RDM KNS KGGS KCP*

35

H. At3g02885 (GASA5)

5 1 cgctttctat tacactttt tttctttta gtcgcacttc acaatttagct taattaattt
 61 61 cctaaactcg cttattttcc cttttctata tacagatatt atcattagtg acattttcat
 121 121 tttccaaaca gagcgtttag acactagtca actacacaat ataattttcc aattttact
 181 181 gagagaaatg tttttttttt ttttttccaa ggcaagagg tagtctttt gttctctata
 241 241 cgtggtaat tagtgattag taattttacac tggtagtct ttgacattgt ctaagagaca
 301 301 aaaacgacaa gtgtggtagc taatttagaaa taaaatgac ctacttcccc agaatcacgg
 361 361 catgaacatt ggcacatcca aatttcttga atccatggc aggaatccaa cactaatcat
 421 421 tttctctata aataatcttta atccgttttta ttgtttctta agaattttttt attggcaatc
 481 481 aagattttt aaccaaaaaa ATGGCGAATT GTATCAGAAG AAATGCTCTT TTCTTCTTGA
 541 541 CTCTTCTCTT TTTATTGTCA GTCTCCAACC TCGTTCAAGgt aaaccactca aaacagattc
 601 601 agtttattaa agtctgtat tgaagtttt tatattacag gctgcgtgt gggtaaaaaa
 661 661 tgaccaaagg ctatacatcc cttaaaaaatt taatggctat tagttttctgt atattgaagt
 721 721 tttatataata tatacagagGC TGCTCGTGT GGTGGCAAC TCAAAACCCCA ACgtacggac
 781 781 tcaaaacttt tggtgtttca tatgtatcata ttaatttttta aatcactaat tattgtataat
 841 841 gttgataaaat aaactttaaa gtaacaataa tggtgtttt tttgtgaaat gtcagttttc
 901 901 tagtatactg tagtctgtga attataagca tgaacataaa gatctcaatg atttggttt
 961 961 tgtttgggg tttgtatgt ctttttgtat gggaaacttca attgttagAGT GCAACTCAAA
 1021 1021 GTGTAGCTTC CGTTGTTCA CGAACATCACA CAAGAAGCCA TGCATGTTCTT TTGCCTCAA
 1081 1081 GTGTTGCAAA AAATGCTTTT GTGTTCTCC TGGCACTTTC GGCAACAAAC AAACCTGTCC
 1141 1141 ATGTTACAAC AACTGGAAGA CAAAGAAGG CCGTCCAAAA TGTCTTAAacttctttt
 1201 1201 agatatattt gataatattt atctgtttt ggattatcaa acacttacta ctctgtttta
 1261 1261 atctgttttca acaagtggc gatttgctc tacactttt ttgtgtcttt tgctcttaac
 1321 1321 tgttgtttt gttatacgtg taagccgc caatgtgtca tggccgaact tattatggtt
 1381 1381 acatattttt gaaatgggct tcattatcaa ttgatttgag cttacaaaaa ttttttttttttt
 1441 1441 aagccccatca agttgtattt gttatattt cttttttttt ttttttttttttttttttttt
 1501 1501 atttatctct agtgttgatg atgtttgtat gtggaaatgtca ttttttttttttttttttttt
 1561 1561 tttaaaaaacc atcaacttgc taaggtaaaa ttctaatattt actgtgaaaaa acattattt
 1621 1621 cgtgcgtat tataatgtat tataatgtat ttttttttttttttttttttttttttttttttt
 1681 1681 tatgtcaaa

35

MANCIRRNALFFLTLLFLLSVSNLVQAA

40 RGGGKLIKPOQ

CNSKCSFRCSATSHKKPCMFFCLKCKKCLCVPPGTGKQTCPCYNNWKTKEGRPKCP*

45

I. At4g09600 (GASA3)

1 taggctggca atttaactct gagacgttct tcttgtatag agaataaaac atacgcgtgt
 61 aaaagaaaaac gcgtaatcg aatgtatgtt gttaacgttc gatcgagatg ccaccaaatac
 121 ttttcattaa aatgaattgt ggaggacata ccactttaa cgaggtcatt tcactgggt
 181 gacatgtgga ctctactttg ggtggcatgt tcataatctt ccacatcacc atgtaaacgt
 241 gaaaacaccc accacactca ttacatctc aaacacatgt ttacgttatac gtacgtatgt
 301 ccaaaaaaaa aatgtaaaaac taggttttagt gattctttt cgcaatgtat aatataacaac
 361 ttgtaaaaat aaaaatatttg aataaggattt ataaataaaac ccaaagaggt gtttagattt
 421 tatacttaat tgttagctact aataagagaa tcagagagaa tagttttata tcctgcacga
 481 aactgcattgc tttttgagac ATGGCAATCT TCCGAAGTAC ACTAGTTTA CTGCTGATCC
 541 TCTTCTGCCT CACCACTTTT GAGgttccata acttttgctt ttacttctcc atgaatcatt
 601 tgcttcgtctt tattcttaat tcataatgtt ttgtatcaatg ataaataattc atcattctct
 661 tcagCTCAT GTTCATGCTG CTGAAGATTC ACAAGTCGGT GAAGGGTAG TGAAAATTGg
 721 tatgttaacgc taacatataat gtaaaagtgtt atatctctgt ttatataatgat tttttaaacg
 781 gttaaaaact agtcatatgt gtatataat atcatgttga gATTGCGGTG GGAGATGCAA
 841 AGGTAGATGC AGCAAATCGT CGAGGCCAAA TCTGTGTTG AGAGCATGCA ACAGCTGTTG
 901 TTACCGCTGC AACTGTGTGC CACCAAGGCAC CGCCGGAAAC CACCACCTTT GTCCCTGCTA
 961 CGCCTCCATT ACCACTCGTGTG GTGGCCGTCT CAAGTGCCTT TAAacatata cacatacaga
 1021 tgtgttata tgcgttccgc gagcacacac gtacgtttat gttttaaagga caatagtatg
 1081 tatgaggcgc tataaacaacaa ccagaaggta atggttcatg ttgaactagt ataagggtta
 1141 tgaactgtgc ttctttgaa caaccacttt tgctgttaatg tttagcaaccc tatttaataa
 1201 attagagatt acaaaaaaaa aaatgaaaaaa tgttttaaaaaa acgtggattt tttaaatttgg
 1261 gattaaaaat taatttcat ttgggtttagt ttgtcaataa attagactaag ttttgtatac
 1321 tagggccgttt aagatatgtctt tgataataga gttgccttag aagttcataa
 1381 ctgttaatataat ctaacttcac ttcaatctca caaacaacacg aatcaacttc agactaaga
 1441 atcgaatttga ccagaacttga aagaaaagttaa aatcatgttga aatataacgaa

30 MAIFRSTLVLLILFCLTTF

ELHVHAAEDSQVGEVVKID

35 CGGRCKGRCSKSSRPNLCLRACNSCCYRCNCVPPGTAGNHLCPCYASITTRGGRLKCP*

J. At4g09610 (GASA2)

1 ttaacagttt aacaccataa tggttaaactc gggttagcat tttgggtgtaa ttcttacccct
 5 61 ttaaccatac atactaaaga cgcagagaag ttcatatggt agttaatcgt aaatagctaa
 121 acttttaatt ggggttaaca tattattna cacttaacat ttaacttattg atctctcatt
 181 ttttttttat taaccaaaat aaattcattt tagaaccaaa cgtttcaaaa actcgtaatg
 241 ttttctcatt aaatcttac tatagctcac acaaagaaaa actacggaca tgcacatgcacc
 301 caattatata catggattat tatttttagt gttataatata gataccaaaat aaaaaacatt
 361 tggatagccg ataggcgata gccactataa atataccaaa gaggttggat tatacatata
 421 ggcgttaatac caaagagagt atcagataga aatagttcta atattttgtt caactcacag
 481 aaattgcattt agtttcgaaac ATGGCAGTC TCCGAAGTAC ACTGGTTCTG TTACTAATCA
 541 TCGTCTGTCT CACCACTTAT GAGGtttata atatttttgg tctttatagt tccccaaagaa
 601 cacctagcaa tattatactc aattcatgtt tataatgttata tgactgtatca ttctcttcag
 661 CTTCACGTCC ACGCTGCTGA TGGTGCAAAG GTCGGTGAAG GCGTAGTGAATGAA ATCGGtATG
 721 taaccctaac ttatataataa cacgttggta tataacttaa tattttgtat ggggtcactc
 781 ttttcccaac ttatataataa ctttggatgg gagaatgttca aagctttta atgagatgtt
 841 atatctccga gaaggaaact atgaacttaa agctttggat tcccttgcaaa caaatataaaa
 901 cttttgatgg gttttaaacgg attaaatttag ttacatgtgt ttgtatgaatg tatgtatgt
 961 tggtagATTGT GGTGGGAGAT GCAAAGATAG ATGCAGCAAA TCTTCGAGAA CGAAGCTATG
 1021 CTTGAGAGCG TGCAACAGCT GTTGTCCCCG CTGCAACTGT GTGCCACCTG GTACTTCTGG
 1081 AAACACCCAC CTTTGTCTT GCTACGCCCT CATTACCACT CACGGTGGCC GCCTCAAGTG
 1141 CCCTTAAtt ttcttctgtg tctgtttctg tttctacttc tatttcaat atatgtatcat
 1201 gtgtgtgtac gtgtgtatgt atacaagtac tgctatgtt tggaggacaa aagtatatgt
 1261 atgagaagct ataaactaat tagaagtgtt tgggttatgcg tattatcaaa ccgtgttact
 1321 tctgaacaaac caatttcgggt ttgttccaaag ttggcaacc ctaaaaataaaa aattcaaaaat
 1381 gattggagac tactcgtaa tagacattgtt aaacgtatgtt atctcggttac gttttatata
 1441 tttttgaact gtaatattat tatgcagaag cgggtttgtt atggccgac aaaaaaaaaaag
 1501 tggttttgtt atggatatgtt ttcggatcta ttctggaaat ggtctcaaaa agtagagtt
 1561 agatctcaat acgaaaatgtt accctttcggtt ttgatttatac aaagcctttt attttggaaa
 1621 cgtttaatcc tcacttaggtt ctctctt

35 MAVFRSTLVLLIIVCLTTY

ELHVHAADGAKVGEGVVKID

CGGRCKDRCSKSSRTKLCLRACNSCCSRCNCVPPGTSGNTHLCPCYASITTHGGRLKCP**

K. At5g15230 (GASA4)

MAKSYGAIFLLTLIVLFMLQTMV

40

MASSGSNVKWSQKRYGPGSLKRTQ

CPSECDRRCKKTQYHKACITFCNKCCRKCLCVPPGYYGNKQVCSCYNWNKTQEGGPKCP**

45

L. At5g14920

5	1	tttgcactgt	gtgcataata	cgaagtgaag	aggcttttta	tatgaaat	ataagcgaca
	61	cagccttatg	ggcaaatacg	atgctatttta	tttattttgtat	aagaagatta	ataatttcaaa
	121	tttgcatcc	actagtctct	tgggttactc	aaaacatata	acccaaaaagt	ccatagattt
	181	atttgttctt	attactgtt	aaagtatttcc	aagtgtatgt	acgaaaaaaag	tggcaatttcc
	241	atgttatttac	aatataatcc	atttttggga	atgtatattt	ttgttattatc	ttgagcttgc
	301	agagatata	tttggtgcc	tgaaggttca	aagctggcat	gcatatgtca	tataataact
10	361	gctctggacc	taataacttac	tacgcatttta	aattaatattt	tatggataat	atgggttataa
	421	aataaggaac	ttcttatttta	atcacaaaag	gtcaactggtc	ttcttcgtgt	gacttcacca
	481	ctttcttcata	ccccacaaaa	ATGGCTCTCT	CACTTCCTTC	AGTCTTTATC	TTTTCCATG
	541	TCTTTACCAA	Tgtaaagtat	tcttactttt	cataacaaaa	gggttattta	tgttaaagac
	601	tacataatata	tatacaattta	tgtgcattac	gttttgcgt	attgtacta	actatgtatt
	661	ttgttattatc	accgagcagG	TTGTTTTGTC	TGCTTCAAAAT	GAGGAATCCCA	ACGCCTTATGt
15	721	acgttttctta	atttccagtt	taatttatttc	tatgcgttctt	taactatata	ctcaggcatt
	781	tttatttgatt	atttgtatgt	aagttaaattt	tttgtatatgt	tttgttattaa	atttataatGT
	841	TTCCTTTACCA	ACGCCAACAC	TTCCATCGCC	ATCTCCGGCT	ACCAAACCCG	CGTCGCAGC
	901	TCTCAAAACCG	CCGACGCCGT	CGTCAAGGCC	ACCCACGCTG	CCAAACTACT	CTATTAAACCC
	961	ACCCACCAAC	AAACCTCCCG	TCAACACCTCC	AACTATTCCG	GTTACACAGC	AAAAACCTCC
20	1021	GGTTTCAACT	CCTCCGATCG	AACTACCGCC	GGTAAACCCA	CCTACGTACA	AACCCCCAAC
	1081	GCCAACAGTT	AAACCACCGT	CCGTCACCC	ACCTACGTAC	AAACCCCCAA	CTCCAAACGGT
	1141	TAAACCACCC	ACTACATCAC	CGGTTAAACC	ACCCACTACG	CCACCAGTTC	AATCACCGCC
	1201	GGTCCAACCA	CCTACGTACA	AAACCCCCAAC	GTCAACCGGTT	AAACCCACCA	CCACAACTCC
	1261	ACCGGGTTAAA	CCCCCCCCCA	CGACGCCAC	GGTCCAACCA	CCTACGTACA	ATCCCCAAC
	1321	TACACCGGGT	AAACCAACCTA	CAGCGCCGCC	TGTCAACACT	CCAAACACCAC	CTCCCGTAAG
	1381	AACTCGGATA	tgataataat	attttttttc	aaaagtgtga	tgattatcgg	tggttgtatta
	1441	gatcgatgt	ataattggac	taaattttgg	acggtttagA	TTGCGTGCCT	TTATGTGGGA
	1501	CGAGGTGTGG	GCAACACTCG	AGGAAGAACG	TATGTATGAG	AGCGTGCCTC	ACGTGCTGCT
	1561	ACCGCTGCAA	GTGTGTTCCC	CCAGGACACCT	ACGGTAAATAA	GGAGAAGTGT	GGATCTGTGTT
	1621	ACGCCAACAT	GAAGACACGT	GGTGGAAAAT	CCAAATGTCC	TTGAaccctt	atagcagat
	1681	ggttgtaaaa	cgaataataat	taaataatgt	gagttttttt	aaagggtgtaa	ttgttgcgtt
	1741	tttgttata	taatatttgg	ttggatcttt	gttacgggaa	cgtagaatac	taaataatgt
30	1801	aaaaaacctt	ctcgatgaat	taagggtttt	atgaatttgt	tttgttattga	ataatataatgg
	1861	gatggataaa	gttttattat	tctaacaggt	tactttttt	ggcattttctt	cggctcatgt
	1921	aactttgtt	tgcgttacac	tatgtatatgt	atagaagaac	ctaaaaaaag	aaagaaaaaca
	1981	agaaaatgtc	atagcgaac	tcaaaaatgt	agttttctgc	tagcgttaat	ttgttatttgc
	2041	agttgggttca	aatgtcttac	ttgcataatct	tattttggcc	ttatataatgc	tttatgttgc
	2101	atatgttcc	gccttatttgg	gcgcgtgtgt	ttgaagatca	tttggaaag	tcttgcgcac
40	2161	ggag					

MALSLLSVFIFHVFTNVVFAAS

45 NEESNALVSLPTPTLPPSP
TKPPSPALKPPTPSYKPPPTLP
TTPPIKPPPTKPPVKPPTIPV
PVKPPVSTPPPIKLPPVQPPTY
KPPTPTVKKPSVQPPTYKPP
50 PTVKPPTTSPVKPPTPPVQS
PPVQPPTYKPPTSPVKPPTTT
PPVKPPTTTPPVQPPTYNPPT
TPVKPPTAPPVKPPTPPPVRT
RID
55 CVPLCGTRCGQHSRKNVCMRACVTCCYRCKCVPPGTYGNKEKCGSCYANMKTRGGKSKCP*

M. At5g59845

1 gacttgagta tgaatccaat aacccaaaat ttatgcagat tttagaatac ttcttataaa
5 61 tcttaaatga ataacacaaa actttaacat acttttaaca aatcttgatt gaataacaac
121 agatttaca tgacattta aatcaaaaa actctttga aatcataaaac caataacaac
181 cccttagttt ttactatTTT gaattctgac gtactttttt attagttgaa ttcttataaa
241 tgagaaaaaca ttaatttattt ctaatctttt gaacttaagg cccacaaaaa tcttataaaat
301 tgggacagat ggactagata acaagcggtt cacctactcc aaaatttccc tataagtaac
361 tctttttgtt acctcctttt ctccccaaac catcactctt ttgcattgt gtgaaaccc
421 cgagtttttcttcatcttc tcaagtaac aaacttttc caaacagatt attattaaaa
481 caatctcata aagaactacg ATGAAATTCC CGGCTGTAAG AGTTCTTATT ATCTCTCTTC
541 TCATCACATC TTCTTGTTC ATACTCTCAA CCGCGGATTG GTgttaagtat acacaatgca
601 ttttctttttt ttagataactt ttcttcattag aaatttagct ttcttaataa aattgtattg
661 ttagatggta ttaatttagCA CCATGCGGAG GAAAATGCAA CGTGAGATGT TCAAAGGCAG
721 GAAGACAAGA TAGGTGTCTC AAGTATTGTA ATATATGTTG CGAGAAGTGT AACTATTG
781 TTCTTCAGG CACTTATGGA AACAAAGATG AATGCCCTTG TTACCGCGAT ATGAAGAACT
841 CCAAAGGCAC GTCCAAATGT CCTTGAtcat gttcttaaga ttatccttat agacacaata
901 tcttggaaatg ttaagattgt gtttgatgca taaaataatg agcttgagat acttctatga
961 atgaatatatgt gaaagatttt gacaataaaa tgatttgatg tattaaaata ttcttagtga
1021 agttatatatgt gtataaatga agtataatgat atacattgtt tggtgcattt catgagaaaag
1081 ataaatctac aacaatccaa tttatggaaa ttttactaag ttaactgtatc aaaaacgtta
1141 attatgtttt agaatcttgc tgagagatga ttactttttt aagagaaaatt gattgtttgt
1201 tgtcaatgag gataaaagtaa gaagccattt ctcaacacat ggacttgata gcaaactaaa
1261 caaggctcaa gcattgaaat tggaaacgtct cgatagataa gatggctca agaaaagcaa
1321 gtgtttttt tttttttttt cagaaaatttga aattactgtc tacttt

30 MKFPAVKVLIISLLITSSILFILSTA

DSSP

CGGKCNVRCSKAGRQDRCLKYCNICCEKCNYCVPSGTYGNKDECPCYRDMKNKGTSKCP*

35

N. At3g10170

genomic structure before splicing and processing 5' - towards 3'
predicted orf sequences are underlined

5 CTGTTTCAGAAAATGGCAACAAAACCTAGCATCATTGTTTCTCCATTG
TTGTGTACATCTCTCTGTCTGCCATATGCATGTAAGTGTGTTCAACA
CTCTATTCCCTATGTTCACATTATCAACTTATCTTATACGTCCCTGA
10 ATAAAACACAGCCTATATACTTGAATCTCTGCTCGACAACCACAAACCA
CCACAGTCGCAACCACAACCGCCATCACAAATAACTCTCAAGTGAGTT
CTCGGTTCATCACTACTCAAAAAAAGAGTTTCATGAATCTACAAAACCT
TTTAACATCTTGCATCTCTGTGATTGGCAGTACGGTACTACT
15 CAAGGCAGTCCTCAACCCCAAGGTAAACCCACTGACTAGCCTAGTTTTA
ATTAATGTTTGTGCTGAATGCGAAACTAAATCCGCTATTCCACCTTTATT
AGAGTGCGGGCCAAGGTGTGGAGATAGATGCTCGAATACACAATAAGA
20 AGCCGTGTTGTTCTCTGCAACAAATGTTGAACAAGTGCTTGTGTTG
CCCCCAGGTACTTATGGCAATAAGCAAGTATGTCCTTGCTATAACAACTG
GAAGACCAAGAGCGGTGGACCAAAATGCCCTAGTTCTCTCTTAATT
CTTTAGCATAAAACTCCATGTAATTGTTAATCTACCTATCATAATTATA
TATGTATTGGACTCTCCATAATCAGTTCTGTGATTATGACGT

Amino acid sequence of the predicted pre-pro-peptide
the first line represents the signal sequence
the second (set of) lines represents the the pro-peptide
the last line represents the conserved Cysteine motif.

MATKLSIIVFSIVVLHLLL SAHMH

FLINVCAECETKSAIPPLIE

30 CGPRCGDRCSNTQYKKPCLFFCNKCCNKCLCVPPGTYGNKQVCPYCYNWKTSGGPKCP*

They consist of an N-terminal signal peptide, followed by a variable domain (involved in mobility or cell wall attachment)

5 and a C-terminal domain with 12 conserved cystein residues.

The consensus of this last domain is:

C-C-RC-----C---C--CC- (R/K) C-CVP (P/S) GT-G (N/H) ---C-CY-----G--KCP*

(-) = any amino acid;

(C) = conserved C-residue

10 (/) = either one or the other amino acid at this position;

* = stopcodon

Some members of this gene family have been described

previously, and represent the GASA family in *Arabidopsis*

15 *thaliana* (Plant Mol. Biol. 36 (1998)). Similar family

members containing the same structural motifs are present in rice (like GASR1) and tomato (Plant Journal 2 (1992) 153-159;

Mol. Gen. Genet. 243 (1994) Taylor and Scheuring). In

Arabidopsis, the GASA gene family represents 14 different

20 members, similar as the number for the RKS gene family. Our data on the similar phenotypes for RKS4 and GASA3 (figure 6) and the fact that there are similar numbers of ligands and receptors suggest that there is a single GASA ligand molecule interaction with a single RKS molecule. T-DNA knock out

25 phenotypes observed with several of the other GASA peptide ligand genes also show modifications of organ and plant size like the appearance of extreme dwarf plants resembling brassinosteroid insensitive mutants. Co-localization of RKS genes and GASA ligands on the genome (see figure 4) could

30 provide clues of molecular interactions between GASA molecules and RKS molecules (similar as for S locus proteins and S locus receptor kinases).

Furthermore, in the chapter discussing the effects of roots in RKS transgenic plants, it was shown that overexpression of RKS

35 genes can result in the formation of lateral roots (figure

26). One of the GASA ligands is involved in the formation

and/or outgrowth of lateral roots as discussed in Mol. Gen.

Genet. 243, 1994, 148-157.

Intracellularly, this signal is transmitted onto membrane (but not necessarily plasma membrane) associated NDR-NHL proteins. At least some of the functions of the syntaxin-like NDR-NHL proteins would thereby result in the regulation of vesicle transport and /or the positioning of new cell wall formation. Neighboring cells are known to influence and determine the developmental state and the differentiation of cells. In transgenic plants with RKS and / or NDR-NHL expression cassettes the positioning of new cell walls is modified, resulting in abnormal neighboring cells, resulting in abnormal development of groups of cells like flower meristem primordia as observed and shown with RKS0, RKS13 and NHL10.

Table 2 overview of accessions numbers of RKS signal complex genes in *arabidopsis* and in rice:

	Gene code	contig	gene prediction in At database	<i>Oryza sativa</i> japonica contig	approximate position in bp around:
5	RKS0	At1g71830	f14o23	ok	52.000
	RKS1	At1g60800	f8a5	ok	60.000
	RKS2	At5g65240	mgn23	ok	8000
	RKS3	At5g63710	mbk5	ok	see rks2
	RKS4	At2g23950	t29e15	wrong, exon missing	35.000
	RKS5	At5g45780	mra19	wrong, exon missing	102.000
	RKS6	At5g10290	wt e 23	ok	see rks2
	RKS7	At5g16000	ku e 24	ok	P0038C05
10	RKS8	At1g34210	f23m19	ok	90.000 & 1000 2
	different genes!				
	RKS10	At4g33430	en d 25	wrong, exon missing	see rks0
	RKS11	At4g30520	wu d 20	wrong, exon missing	see rks4
	RKS12	At2g13800	f13j11	wrong, exon missing	see rks10
	RKS13	At2g13790	f13j11	ok	P0633E08
	RKS14	At3g25560	mw12	wrong, exon missing	OSJNBB0015G09
	ELS1	At5g21090	ch e 52	ok	P0003H10
20	ELS2	possibly allelic variant of ELS1	no genomic sequence identified yet	see els1	53.000
	ELS3	At3g43740	by c 21	ok	P0468B07
					52.000

Homology between aa sequences from *arabidopsis* proteins are compared with the rice databases using:
http://mips.gsf.de/proj/thal/db/search/search_frame.html
 protein sequences based on *Oryza sativa* japonica contig sequences.

Arabidopsis thaliana ELS1 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

ttactctcaaattcctttcgatttcctctcttaaacctccgaaagctcac
ATGGCGTCTCGAAACTATCGGTGGAGCTCTCGCAGCTTCGTTAACCTAA
CCTTAGCTTGATTCACCTGGTCGAAGCAAACCTCCGAAGGAGATGCTCTA
15 CGCTCTCGCCGGAGTTGACAGATCCAGACCATGTCCTCCAGAGCTGGGAT
CCAACTCTTGTAAATCCTTGTACCTGGTCCATGTCACCTGTAACCAAGACA
ACCGCGTCACTCGTGTGGATTGGAAATTCAAACCTCTGGACATCTGC
GCCTGAGCTGGGAAGCTTGAACATTACAGTATCTAGAGCTCTACAAAAAC
AACATCCAAGGAACTATACCTCCGAACCTGGAAATCTGAAGAATCTCATCA
20 GCTTGGATCTGTACAACAAACATCTTACAGGGATAGTCCCACTTCTTGGG
AAAATTGAAGTCTCTGGTCTTTTACGGCTTAATGACAACCGATTGACGGTC
CAATCCCTAGAGCACTCACGGCAATCCAAGCCTTAAAGTTGTGACGTCTC
AAGCAATGATTGTGTGGACAATCCCACAAACGGACCCTTGCTCACATTCC
TTTACAGAACTTGAGAACAAACCGAGATTGGAGGGACCGGAATTACTCGGT
25 CTTGCAAGCTACGGACACTAACTGCACCTGAacaactggaaaaacctgaaaaat
gaagaattgggggtgaccttgtaagaacacttaccactttatcaaataatc
acatctactatgtataatataatgttagtccaaaaaaaaaaaaaaaaaaaa

Predicted amino acid sequence of the *Arabidopsis thaliana* ELS1 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt *et al.* (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 4 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich

repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The last domain might be involved in attachment to other proteins or structures within the cell wall.

5

MASRNYRWELFAASL

TLTLALIHLVEANSEG

DALYALRRSLTDP

10 DHVLQSWDPTLVN

PCTWFHVTCNQDNRVTRV

DLGNSNLSGHLA

15 P ELGKLEHLQYLELYKNNIQGTI
PSELGNLKNLISLDLYNNNLTGIV
PTSLGKLKSLVFLRLNDNRLTGPI
PRALTAIPSLKVVDVSSNDLCGTI
PTNGPFAHIPLQNFENNPRLEGPE

20

LLGLASYDTNCT

Arabidopsis thaliana ELS2 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

10 aaaattactcaaattcctattagattactctttcgaccccgatagctcac
ATGGCGTCTCGAAACTATCGGTGGAGCTTCAGCTCGTTAACCTCAA
CCTTAGCTTGATTCACCTGGTGAAGCAAACCTCGAAGGAGATGCTCTTA
CGCTCTCGCCGGAGTTAACAGATCCGGACCATGTCCTCCAGAGCTGGGAT
CCAACCTTTGTTAACCTGTACCTGGTCCATGTCACCTGTAACCAAGACA
15 ACCGCGTCACTCGTGTGGATTGGGAATTCAAACCTCTGGACATCTTGC
GCCTGAGCTTGGGAAGCTTGAACATTACAGTATCTAGAGCTCTACAAAAC
AACATCCAAGGAACATACCTTCCGAACCTGGAAATCTGAAGAATCTCATCA
GCTTGGATCTGTACAACAACAAATCTTACAGGGATAGTTCCACTTCTTGGG
AAAATTGAAGTCTCTGGTCTTTACGGCTTAATGACAACCGATTGACGGGG
20 CAATCCCTAGAGCACTCACTGCCAATCCAAAGCCTAAAGTTGTGGATGTC
TAAGCAATGATTGTGTGGAACAATCCAAACAAACGGACCTTGCTCACAT
TCCTTACAGAACCTTGAGAACAAACCCGAGGTTGGAGGGACCGGAATTACTC
GGTCTTGCAAGCTACGACACTAACTGCACTGAagaaattggcaaaacctga
aaatgaagaattggggggaccttgaagaacacttcaccactttatcaaat
25 atcacatctactatgtataataagtatatatgttagtccaaaaaaaaatgaa
gaatcgaatagtaatatcatctggtctcaattgagaactttgaggtctgt
atgaaaattaaagattgtactgtaatgttcggtgtggattctgagaagta
acatttgtattggtatggtatcaagttgtctgccttgtctgcaaaaaaaaaa

30

Predicted amino acid sequence of the *Arabidopsis thaliana* ELS2 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as 35 described in Schmidt *et al.* (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 4 leucine residues, each separated by seven other amino acids. The third domain

contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The last domain might be 5 involved in attachment to other proteins or structures within the cell wall.

MASRNYRWELFAASL

ILTLALIHLVEANSEG

10

DALYALRRSLTDP

DHVLQSWDPTLVN

PCTWFHVTCNQDNRVTRV

15

DLGNSNLSGHLA

P ELGKLEHLQYLQLYKNNIQGTI

PSELGNLKNLISLDLYNNNLTGIV

PTSLGKLKSLVFLRLNDNRLTGPI

20

PRALTAIPSLKVVDVSSNDLCGTI

PTNGPFAHIPLQNFENNPRLEGPE

LLGLASYDTNCT

25

Arabidopsis thaliana ELS3 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

10 ttctctccggcgaaaacc**ATGGTGGCGAAACAGTCGGCGGGAGCTTCTAGCAGCTT**
CCCTGATCCTAACCTTAGCTCTAATTCTGCTAACGGAAGCAA**CTCCGAAGGGGACGCTC**
TTCACGCGCTTCGCCGGAGCTTATCAGATCCAGACAATGTTGTTCAAGAGTTGGGATCAA
CTCTGTTAACCTTGACTTGGTTCATGTCACTTGTAAATCAACACCATCAAGTCACTC
GTCTGGATTGGGAATTCAAACATTCTGGACATCTAGTACACTGAACTTGGGAAGCTTG
15 AACATTACAATATCTTGAACTCTACAAAAACGAGATTCAAGGAACATACCTCTGAGC
TTGGAAATCTGAAGAGTCTAATCAGTTGGATCTGTACAACAACAACTCACCAGGGAAAA
TCCCACCTCTTGGAAAATTGAAGCGGCTAACGAAACCGATTGACCGGTCTATTG
CTAGAGAACTCACAGTTATTCAAGCCTTAAAGTTGATGTCTCAGGGAATGATTGT
GTGGAACAATTCCAGTAGAAGGACCTTGAACACATTCTATGCAAAACTTGAGAACAA
20 ACCTGAGATTGGAGGGACCAGAACTACTAGGTCTTGCAGCTATGACACCAATTGCACTT
AAaaagaagttgaagaa

Predicted amino acid sequence of the *Arabidopsis thaliana* ELS3 protein.

25 Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt *et al.* (1997).
At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a
30 leucine zipper motif, containing 2 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each
35 approximately 24 amino acid residues. The last domain might be involved in attachment to other proteins or structures within the cell wall.

MVAQNSRRELLAASL
ILTLALIRLTEANSEG

DALHALRRSLSDP

5 DNVVQSWDPTLVN

PCTWFHVTNCNQHHQVTRL

DLGNSNLSGHVL

10 P ELGKLEHLQYLELYKNEIQGTI
PSELGNLKSLISLDLYNNNLTGKI
P SSLGKLKRLNENRLTGPI
PRELTVISSLKVVVDVSGNDLCGTI
PVEGPFEHIPMQNFENNLRLEGPE

15

LLGLASYDTNCT

Arabidopsis thaliana RKS0 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

10 atttttatttattttactttgttttaatgcta~~atgggttt~~aaaagggtt
atcgaaaaatgagt~~gagttgtgtt~~gagg~~ttgtctgt~~aaagt~~gttaatgg~~tggtat
tttcggaa~~gttagg~~tttctcg~~gatct~~gaagagat~~aaatcaagat~~tcgaa~~at~~ttacca
ttgtt~~gtt~~gaa**A**TGGAGTCGAGTTATGTGGT~~TTAT~~CTTACTTCACTGATCTTAC~~TT~~
CCGAATCATTCACTGTGGCTTGCTTCTGCTAATTGGAAGGTGATGCTTG~~CATACT~~TTG
15 AGGGTTACTCTAGTTGATCAAACAATGTCTTG~~CAGAGCT~~GGGATCCTACGCTAGTGAAT
CCTTGCACATGGTT~~CCAT~~GTCACTTGCAACA~~ACGAGAAC~~AGTGT~~CATAAGAG~~TTGATT~~TG~~
GGGAATGCAGAGTTATCTGCCATTAGT~~CCAGAG~~CTTGGGTG~~CTCAAGA~~ATTG~~CAG~~
TATT~~TGGAG~~CTTACAGTAACAACATAACTGCCCGATT~~CCTAG~~TAA~~CTTGGAA~~ATCTG
ACAAAC~~TTAGT~~GAGTTGGAT~~CTTACTTAA~~ACAGCTT~~CTCCGG~~CTATT~~CCGG~~A~~ATCA~~
20 TTGGGAAAGCTTCAAAGCTGAGATTCTCCGG~~CTTA~~ACAACAA~~ACAGT~~CTCA~~TGGG~~TCA
ATT~~CCTAT~~GTCACTGACCA~~AT~~TTACTACC~~CTTCA~~AGT~~GTTAG~~ATCT~~CAA~~AA~~ACAGA~~
CTCTCTGG~~TT~~CAGTT~~CCTG~~ACA~~ATGG~~CT~~CC~~TT~~CTCA~~CT~~CCAC~~CC~~ATCAG~~TT~~TG~~
AATAACTTAGACCTATGTGGAC~~CTGTT~~ACAAGTCACCC~~ATG~~C~~CTGG~~AT~~CTCCCC~~G~~TT~~
TCTCCTCCACCAC~~CTT~~TATTCAAC~~CTCCCC~~AG~~TTCCACCC~~GAGT~~GGG~~T~~ATGG~~T~~ATA~~
25 ACTGGAGCAATAGCTGGTGGAGTTG~~CTGC~~AGGT~~GCTG~~CTT~~GGCC~~CTT~~GCTG~~CT~~CC~~TGCA
ATAGC~~CTT~~G~~CTGG~~T~~GGCG~~ACGAAGAAG~~CCC~~ACTAGA~~T~~TTCT~~CGAT~~GT~~CC~~CT~~GCC~~
GAAGAAGATCCAGAAG~~TT~~CATCTGGACAGCTCAAGAGG~~TTT~~CTT~~GCGG~~AG~~CT~~ACAA
GTGGC~~GAGT~~G~~ATGG~~TTAG~~TA~~ACAAGA~~AC~~ATT~~TGGG~~CAGAGG~~GTGG~~TT~~GGGAA~~AGTC
TACAAGGGACG~~CTT~~GGCAGAC~~GGAA~~CTT~~GTT~~G~~CTGT~~CAAGAGACTGAAGGAAGAGCGA
30 ACTCCAGGTGGAGAGCTCCAGTTCAAACAGAAGTAGAGAGT~~GATAAGT~~T~~ATGG~~CAGTT~~CAT~~
CGAAAC~~CT~~G~~TTGAG~~ATTACGAGG~~TTCTG~~T~~ATG~~AC~~ACCG~~AC~~CG~~GAGAGATT~~GCTT~~G~~TGT~~T~~AT~~
CCTTACATGG~~CCA~~ATGGAAGT~~GTTG~~CT~~CGT~~GT~~CTCAG~~AGAGAGAGGCCACC~~GTC~~CACAA~~CC~~T
CCG~~CTTG~~ATT~~GGCC~~AA~~CGCGG~~AAGAGA~~ATCGCG~~CT~~AGG~~CT~~CAG~~CT~~GAGG~~TT~~GT~~CT~~TAC~~
CTACATGATCACT~~TGCG~~AT~~CCGA~~AGATCATT~~CACCGT~~GAC~~GTAA~~AGCAGCAAACATC~~CTC~~
35 TTAGACGAAGAATT~~CGAAGCGG~~TT~~GTTGG~~AGATT~~CGGG~~TT~~GGC~~AA~~AGCT~~T~~ATGG~~ACT~~AT~~
AAAGACACTC~~ACGT~~GACAACAGCAGT~~CCGT~~GGCACC~~ATCGG~~T~~CACATCG~~CT~~CCAGA~~AT~~AT~~
CTCTCAAC~~CCGG~~AA~~AT~~CTTCAGAGAA~~ACCGAC~~G~~GT~~TT~~CGG~~AT~~ACG~~GA~~AT~~C~~ATG~~C~~TTCTA~~
GAAC~~TAAT~~CACAGG~~ACAA~~AGAGCT~~TCG~~AT~~CTCG~~CT~~CGG~~TA~~GCTA~~AC~~GACG~~AC~~GACG~~TC
ATG~~TTACTT~~GACT~~GGGT~~GAA~~AGG~~ATT~~GTT~~GA~~AGG~~GAGAAGAAG~~CTAGAG~~AT~~GTTAGT~~GG~~GAT~~
40 CCAGATCTTCAAACAA~~ACTAC~~GAGGAGAGA~~ACTG~~GA~~ACAAGT~~G~~ATACAAGT~~GG~~GCTTG~~

CTATGCACGCAAGGATCACCAATGGAAAGACCAAAGATGTCTGAAGTTGTAAGGATGCTG
GAAGGAGATGGGCTTGGAGAAATGGGACGAATGGCAAAAGTTGAGATTTGAGGGAA
GAGATTGATTTGAGTCCTAACCTAACTCTGATTGGATTCTGATTCTACTTACAATTG
CACGCCGTTGAGTTATCTGGTCCAAGGTAaaaaaaaaaaaaaaaaaa

5

Predicted amino acid sequence of the *Arabidopsis thaliana* RKS0 protein.

Different domains are spaced and shown from the N-terminus

10 towards the C-terminus. Overall domain structure is similar as described in Schmidt *et al.* (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 4 leucine residues, each separated by seven other amino

15 acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline
20 residues, and to be a site for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine protein kinase domain (Schmidt *et al.* 1997) and is probably also
25 containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

30

MESSYVVVFILLSLILLPNHSL
WLASANLEG

DALHTLDRVTLVDP

35 NNVLQSWDPTLVN

PCTWFHVTCNNENSVIRV

DLGNAELSGHHLV

40 P ELGVLKLNQYLELYSNNTGPI

PSNLGNLTNLVSLDLYLNSFSGPI
PESLGKLSKLRFLRLNNNSLTGSI
PMSLTNITTLQVLDLSNNRLSGSV
PDNGSFSLFTPISFANNLDLCGPV

5

TSHPCPGSPPFSPPPP
FIQPPPVS TPSGYGITG

AIAGGVAAGAAL

10 PFAAPAIAFAWW

RRRKPLDIFFDVPAEEDPE
VHLGQLKRFSLRELQVAS

15 DGFSNKNILGRGGFGKVYKGRILAD
GTLVAVKRLKEERTPGGELQFQ
TEVEMISMAVRNLLRLRGFCM
TPTERLLVYPYMANGSVASCLR
ERPPSQPLDWPTRKRIALGSA
20 RGLSYLHDHCDEPKIIHRDVKAA
NILLDEEFEAVVGDFGLAKLMD
YKDTHVTTAVRGTI GHIAPEYL
STGKSSEKTDVFGYGIMLLELI
TGQRAFDLARLANDDDVMLLDW
25 VKGLLKEKKLEMLVDPDLQTNY
EERELEQVIQVALLCTQGSPME
RPKMSEVVRMLE

GDGLAEKWDEWQKVEILREEIDL S

30

PNPNSDWILDSTYNLHAVELSGPR

Arabidopsis thaliana RKS1 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

10 ccaaaggattgattgcttaagaaggat**ATGGAAGGTGTGAGATTGTGGTGTGGAGATTA**
GGATTCTGGTTTGTATGGTCTTGATATCTCTCTGCTACACTTCTCCTACTGGT
GTAAACTATGAAGTGACAGCTTGGTTGCTGTGAAGAA**ATGAATTGAATGATCCGTACAAA**
GTTCTGAGAATTGGGATGTGAATTCAAGTTGATCCTTGTAGCTGGAGA**ATGGTTCTTGC**
ACTGATGGCTATGTCTCTCACTGGATCTTCAGCCAA**AGCTTGTCTGGTACATTGTCT**
15 CCTAGAATCGGAAACCTCACCTATTTACA**ATCAGTGGTGTGCAAAACAATGCAATCACT**
GGTCCAATTCCGAAACGATTGGGAGGTTGGAGAAGCTCAGTC**ACTTGATCTTCGAAC**
AATTCA**TTCACCGGGGAGATACCGGCCTCACTTGGAGAACTCAAGAACTTGAATTACTTG**
CGGTTAA**ACAATAACAGTCTTATAGGAACCTGCCCTGAGTCTCTATCCAAGATTGAGGGA**
CTCACTCTAGTCGACATT^{CG}TATAACA**ATCTTAGTGGTTCGCTGCCAAAGTTCTGCC**
20 AGAACTTC**CAAGGTATTGTAATGCGTTAATCTGTGGCCAAAAGCTGTTCAA**ACTGT
TCTGCTGTTCCCGAGCCTCTCACGCTCCACAAGATGGTCCAGATGAATCAGGA**ACTCGT**
ACCAATGCCATCACGTTGCTTGCATTGCCAGCTCAGTGCAGCATT^{TTT}TGTT
TTCTTACAAGCGGA**ATGTTCTTGGTGGAGATATGCCGTAACAAGCAAATATTTTT**
GACGTTA**ATGAACAATATGATCCAGAAGTGAGTTAGGGACTTGAAGAGGTATACATTC**
25 AAAGAGCTTAGATCTGCCACCA**ATCATTCAACTCGAAGAACATTCTCGGAAGAGGCCGGA**
TACGGGATTGTGTA**CAAAGGACACTTAAACGATGGAACTTGGTGGCTGTCAAACGTCTC**
AAGGACTGTA**ACATTGCCGGTGGAGAAGTCCAGTTCAAGACAGAAGTAGAGACTATAAGT**
TTGGCTCTTCATCGCA**ATCTCCCTCCGGCTCCGGTTCTGTAGTAGCAACCAGGAGAGA**
ATTTAGTCTACCC**TTACATGCCAAATGGGAGTGTGCGATCACGCTTAAAGATAATATC**
30 CGTGGAGAGCCAGCATTAGACTGGT**CGAGAAGGAAGATAGCGGTGGACAGCGAGA**
GGACTAGTTACCTACACGAGCA**ATGTGACCCGAAGATTATACACCGCGATGTGAAAGCA**
GCTAACATTCTGTTAGATGAGGACTTCGA**AGCAGTTGTTGGTGA**TTGGT**AGCTAAG**
CTTCTAGACC**ATAGAGACTCTCATGT**CAAACTGCAGTCCG**TGGA**ACTGTTGCCACATT
GCACCTGAGTACTTATCCACGG**GTCA**GTCC**TAGAGAAGACTGATGTCTTGGCTTGGC**
35 ATACTCTC**TTGAGCTCATTACTGGTCAGAAAGCTTGA**TTTGGCAGATCCGCACAC
CAGAAAGGT**GTAATGCTTGA**CTGGG**TAAGAAGCTGCACCAAGAAGGGAAACTAAAGCAG**
TTAATAGACAAAGATCTAAATGAC**AAAGTTCGATAGAGTAGAAACTCGAAGAAATCGTCAA**
GTTGCG**CTACTCTGCACTCAATTCAATCCATCTCATG**ACCGAAA**ATGTCAGAAGTTATG**
AAGATGCT**GAAGGTGACGGTTGGCTGAGAGATGGGAAGCGACGCAGAACGGTACTGGT**
40 GAGC**CATGCCACCGCCATTGCCACCGGGATGGTGA**GTCTTCGCCG**GTGTGAGGTAT**

TACTCGGATTATTCAGGAATCGTCTCTTGAGTAGAAGCCATTGAGCTCTCGGGTCCT
CGATGAttatgactcactgttttaaaaaaa

5 Predicted amino acid sequence of the *Arabidopsis thaliana* RKS1 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).

10 At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 3 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate

15 bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for O-

20 glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eighth domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably

25 also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

30

MEGVRFVVWRLGFL
VFVWFFDISSATLSPTGVNYEV

TALVAVKNELNDP

35 YKVLENWDVNSVD

PCSWRMVSCTDGYVSSL

DLPSQSLSGT
LSPRIGNLTYLQSVLQNNAITGPI
PETIGRLEKIQSLDLSNNNSFTGEI
PASLGELKNLNYLRLNNNSLIGTC
5 PESLSKIEGLTLVDISYNNLSGSL
PKVSARTFK VIGNALICGPK

AVSNCSAVPEPLTL
PQDGPDESGTRTNG
10 HHVALAFAASFS
AAFFVFFTSGMFLWW

RYRRNKQIFFDVNEQYDPE
15 VSLGHLKRYTFKELRSAT

NHFNSKNILGRGGYGINVKGHLND
GTLVAVKRLKDCNIAGGEVQFO
TEVETISLALHRNLLRLRGFCS
20 SNQERILVYPYMPNGSVASRLK
DNIRGEPALDWRRKKIAVGTA
RGLVYLHEQCDPKIIRDVKA
NILLDEDFEAVVGDFGLAKLLD
HRDSHVTAVRGTVGHIAPEYL
25 STGQSSEKTDVFGFGILLLELI
TGQKALDFGRSAHQKGVMLDW
VKKLHQEGKLKQLIDKDLNDKF
DRVELEIIVQVALLCTQFNPSH
RPKMSEVMKMLE

30 GDGLAERWEATQNGTGEHQPPPLPPGMVSSS

PRVRYYSDYIQESSLVVEAIELSGPR

Arabidopsis thaliana RKS2 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

The first stopcodon has been underlined.

5 Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

Italics indicate the presence of an alternatively spliced gene product.

10

tcaatttggtagcttttagaaaa**ATGGCTCTGCTTATTACTGCCTAGTTAGT**
AGTTTATGGTCATCTGTGTCACCAGATGCTCAAGGGGATGCATTATTGCGTTGAGGAGC
TCGTTACGTGCATCTCCTGAACAGCTAGTGATGGAACCAGAATCAAGTCGATCCTTGT
ACTTGGTCTCAAGTTATTGTGATGACAAGAACATGTTACTCTGTAACCTGTCTTAC

15

ATGAACCTCTCCTCGGGAACACTGTCTTCAGGAATAGGAATCTGACAACACTCTCAAGACT
CTTACATTGAAGGGAAATGGAATAATGGGTGGAATACCAGAATCCATTGAAATCTGTCT
AGCTTGACCAGCTTAGATTGGAGGATAATCACTTAACTGATCGATTCCATCCACTCTC
GGTAATCTCAAGAATCTACAGTTCTCAGGACCTGAGTAGGAATAACCTTAATGGTTCT
ATCCCGGATTCACTTACAGGTCTATCAAAACTGATAAAATTCTGCTCGACTCAAATAAT

20

CTCAGTGGTAGATTCTCAGAGTTATTCAAAATCCAAAATACAATTTCACAGCAAAC
AACTTGAGCTGTGGTGGCACTTCCCGAACCTGTGTAACCGAGTCCAGTCCTTCAGGT
GATTCAAGCAGTAGAAAAACTGGAATCATCGTGGAGTTAGCGGAATAGCGGTTATT
CTACTAGGATTCTTCTTTCTCTGCAAGGATAAACATAAAGGATATAAACGAGAC
GTATTGTGGATGTTGCAGGAACGAACCTTAAAAAGGTTGATTCAGGTGAAGTGGAC

25

AGAAGGATTGCTTTGGACAGTTGAGAAGATTGCATGGAGAGAGCTTCAGTTGGCTACA
GATGAGTTCACTGAAAAGAATGTTCTCGGACAAGGAGGCTTGGAAAGTTACAAAGGA
TTGCTTCGGATGGCACCAAGTCGCTGTAAAAAGATTGACTGATTGAAACGTCCAGGA
GGAGATGAAGCTTCCAGAGAGAAGTTGAGATGATAAGTGTAGCTGTTAGGAATCTG
CTTCGCCTTATCGGCTTTGTACAACACAAACTGAACGACTTTGGTGTATCCTTCATG

30

CAGAACATCTAAGTGTGCATATTGCTTAAGAGAGATTAAACCCGGGGATCCAGTTCTGGAT
TGGTCAGGAGGAAACAGATTGCGTTAGGTGCAGCACGAGGACTCGAATATCTCATGAA
CATTGCAACCCGAAGATCATACACAGAGATGTGAAAGCTGCAAATGTGTTACTAGATGAA
GACTTGAAGCAGTGGTTGGTGATTTGGTTAGCCAAGTTGGTAGATGTTAGAAGGACT
AATGTAACCACTCAGGTCCGAGGAACAATGGGTCAATTGCACCAGAATGTATATCCACA

35

GGGAAATCGTCAGAGAAAACCGATGTTTGGGTACGGAATTATGCTTCTGGAGCTTGT
ACTGGACAAAGAGCAATTGATTTCTCGCGGTTAGAGGAAGAAGATGATGTCTTATTGCTA
GACCATGTGAAGAAAAGTGGAAAGAGAGAAGAGATTAGAAGACATAGTAGATAAGAAGCTT
GATGAGGATTATATAAAGGAAGAAGTTGAAATGATGATAACAAGTAGCTCTGCTATGCACA
CAAGCAGCACCGGAAGAACGACCAGCGATGTCGGAAGTAGTAAGAATGCTAGAAGGAGAA

40

GGGCTGCAAGAGAGATGGGAAGAGTGGCAGAATCTGAAGTGACGAGACAAGAAGAGTTT

CAGAGGTTGCAGAGGAGATTGATTGGGTGAAGATTCCATTAATAATCAAGATGCTATT
GAATTATCTGGTGGAAAGATAGaaacaaaaaa

5 Predicted amino acid sequence of the *Arabidopsis thaliana* RKS2 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).

10 At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 3 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate
15 bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 3 complete and 2 incomplete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site
20 for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eighth domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably
25 also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions. Italics indicate an alternatively spliced gene
30 product.

MALLIITALVFSSL

WSSVSPDAQG

35 DALFALRSSLR

ASPEQLSDWNQNQVD

PCTWSQVICDDKKHVTSV

TLSYMFSS GTLSSGI
G ILTTLKTLKGNNGIMGGI
PESIGNLSSLTSLDLEDNHLDRI
5 PSTLGNLKNLQFLTLSRNNLNGSI
PDSLTGLSKLINILDSNNLNGEI
PQSLFKIPKYN FTANNLSCGG

TFPQPCVTTESSPSGDSSSRKTG
10 IIAGVVSGIAVIL
LGFFFFFC

KDKHKGYKRDVFVDVAGTNFKKGLISGE
15 VDRRIAFGQLRFAWRELQLAT

DEFSEKNVLGQGGFGKVKYKGLLSD
GTKVAVKRLTDFERPGGDEAFQ
REVEMISVAVHRNLLRLIGFCT
20 TQTERLLVYPFMQNLCSVAYCLR
EIKPGDPVLDWFRRKQIALGAA
RGLYLYLHEHCNPKIIHRDVKAA
NVLLDEDFEAVVGDFGLAKLVD
VRRTNVTTQVRGTMGHIAPCI
25 STGKSSEKTDVFGYGIMLLELV
TGQRAIDFSRLEEEDDVLLDH
VKKLEREKRLEDIVDKKLDEDY
IKEEVEMMIQVALLCTQAAPEE
RPAMSEVVRMLE

30 GEGLAERWEEWQNLEVTRQEEFQ

RLQRRFDWGEDSINNQDAIELSGGR

35

Arabidopsis thaliana RKS3 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

The first stopcodon has been underlined.

5 Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

aacggtgaaaagtccatgatcctttcgaggattcattcaaagaaattgttttagatgg
10 aacaatcagaaattgtatcttacaatgtttc**ATGGCCTTAGCTTTGTGGAAATCACTTCG**
TCAACAACTCAACCAGATATCGAAGGAGGAGCTCTGTTGCAGCTCAGAGATTGCTTAAT
GATTGAGCAATCGTCTAAATGGACACGCGATTGTGAGCCCTGCTATAGTTGGTCT
TATGTTACCTGCAGAGGCCAGAGTGTGAGCTCTAAATCTGCCTCGAGTGGATTCA
GGAACACTCTCCAGCTATTACAAAATCTGAAGTTCTGGTTACCTAGAGTTACAGAAC
15 AATAGTTATCTGGTGCCTTACAGATTCTCTGGGAACATGGTTAATCTACAGACTTTA
AACCTATCAGTGAATAGTTCAGCGGATCGATACCAGCGAGCTGGAGTCAGCTCTCGAAT
CTAAAGCACTGGATCTCATCCAATAATTAAACAGGAAGCATCCAACACAATTCTC
TCAATCCAACATTGATTTCAGGAACTCAGCTTATATGCGTAAAGTTGAATCAG
CCTTGGTCTTCAAGTTCTCGTCTTCCAGTCACATCCTCCAAGAAAAGCTGAGAGACATT
20 ACTTTGACTGCAAGTTGTGTTGCTTCTATAATCTTATTCTGGAGCAATGGTTATGTAT
CATCACCATCGCGTCCGCAGAACCAAATACGACATCTTTTGATGTAGCTGGGAAGAT
GACAGGAAGATTCCCTTGGACAACCTAAAACGATTCTCTTACGTGAAATCCAGCTCGCA
ACAGATAGTTCAACGAGAGCAATTGATAGGACAAGGGAGATTGGTAAAGTATACAGA
GGTTTGGTCCAGACAAAACAAAAGTTGCAGTGAAACGCCCTGCGGATTACTTCAGTCCT
25 GGAGGAGAAGCTGTTCAAAGAGAGATTCAAGCTCATAAGCGTTGGGTTCAAAAAAT
CTCTTACGCCATTGGCTCTGCACAACCTCCTGAGAGAAATCCTGGTTATCCATAC
ATGGAAAATCTTAGTGTGCTATCGACTAAGAGATTGAAAGCGGGAGAGGAAGGATTA
GACTGCCAACAGGAAGCGTGTAGCTTGGTCACTCACGGTTAGAGTATCTACAC
GAACATTGTAACCGAACGATCATACACCGCGATCTCAAGGCTGCAAACATACTTTAGAC
30 AACATTTGAGCCAGTTCTGGAGATTCGGTTAGCTAAGCTTGTGGACACATCTCTG
ACTCATGTCACAACCAAGTCCGAGGCACAATGGTCACATTGCGCCAGAGTATCTCTGC
ACAGGAAAATCATCTGAAAAACCGATGTTTGGTTACGGTATAACGCTTCTGAGCTT
GTTACTGGTCAGCGCGCAATCGATTTCACGCCGGTGAAGAAGAGAAAATATTCTCTG
CTTGATCATATAAGAAGTTGCTTAGAGAACAGAGACTTAGAGACATTGTTGATAGCAAT
35 TTGACTACATATGACTCCAAAGAAGTTGAAACAATCGTTCAAGTGGCTTCTGCACA
CAAGGCTCACCAGAACGATAGACCAGCGATGTCAAGTGGTCAAATGCTCAAGGGACT
GGTGGTTGGCTGAGAAATGGACTGAATGGAAACAACCTGAAGAAGTTAGGAACAAAGAA
GCATTGTTGCTTCCGACTTTACCGGCTACTTGGGATGAAGAAGAAACCACCGTTGATCAA
GAATCTATCCGATTATCGACAGCAAGATGAagaagaaacagagagagaaagatatctatg
40 aaaa

Predicted amino acid sequence of the *Arabidopsis thaliana* RKS3 protein.

5 Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).
At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a
10 leucine zipper motif, containing 3 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 4 complete repeats of each
15 approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular
20 domains are positioned. The seventh domain has an unknown function. The eighth domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth
25 domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

MALAFVGITSSTTQPDIEG

30

GALLQLRDSLNDSSNRL

KWTRDFVS

PCYSWSYVTCRGQSVVAL

35

NLASSGFTGTL

P AITKLKFVLTLELQNNNSLSGAL

PDSLGNMVNLQTLNLNVNSFSGSI

PASWSQLSNLKHLDSLSSNNLTGSI
PTQFFSIPTFEFSGTQLICGKS

5 LNQPCSSSRLPVTSSKKLRD

ITLTASCVASIIL
FLGAMVMYHHH

10 RVRRTKYDIFFDVAGEDDR
KISFGQLKRFSLREIQLAT

DSFNESNLIGQGGFGKVYRGLLPD

15 KTKAVKRLADYFSPGGEAAFQ
REIQLISVAVHKNLLRLIGFCT
TSSERILVYPYMEMNLSVAYRLR
DLKAGEEGLDWPTRKRVAFGSA
HGLEYLHEHCNPKIIHRDLKAA
20 NILLDNNFEPVLGDFGLAKLVD
TSLTHVTTQVRGTMGHIAPEYL
CTGKSSEKTDVFGYGITLLELV
TGQRAIDFSRLEEEENILLD
HIKKLLREQRLRDIVDSNLTTY
25 DSKEVETIVQVALLCTQGSPED
RPAMSEVVVKMLQ

GTGGLAEKWTEWEQLEEVRNKEALLL

30 PTLPATWDEEETTVDQESIRLSTAR

Arabidopsis thaliana RKS4 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

10 tcttccttctccttctggtaatctaattctaaagctttc**A**TGGTGGTGAAGATATTCTCTGTTCTGTTACTATGTTCTCGTTACTTGTCTCTCTGAACCCAGAAC
CCTGAAGTGGAGGC**G**TTGATAAACATAAAAGAACGAGTTACATGATCCACATGGTGTTC
AAAAACTGGGATGAGTTCTGTTGATCCTGTAGCTGGACTATGATCTCTGTTCTCA
GACAACCTCGTAATTGGCTTAGGAGCTCAAGTCAGTCTCTTCAGGAACCTTATCTGGG
15 TCTATTGGAAATCTCACTAATCTCGACAAGTGTCAATTACAGAACATAACATCTCCGGT
AAAATCCCACC**G**GAGATTGTTCTCTCCAAATTACAGACTCTGGATTATCCAATAAC
CGGTTCTCCGGTGAATCCCCGGTTCTGTTAACCAGCTGAGTAATCTCAATATCTGTTG
AACAAACAACTCATTATCTGGGCCCTTCCTGCTCTGTCTCAAATCCCTCACCTCTCT
TTCTTAGACTTGTCTTATAACAATCTCAGAGGTCCTGTTCTAAATTCCCTGCAAGGACA
20 TTCAATGTTGCTGGAAACCCCTTGATTGTAAAAACAGCCTACCGGAGATTGTTCAGGA
TCAATCAGTGCAAGCCCTCTTCTGTCCTTACGTTCTTCATCAGGACGTAGAACCAAC
ATATTAGCAGTTGCACTTGGTGTAAAGCCTGGCTTGCTGTAGTGTAAATCCTCTCTC
GGGTTCATTTGGTATCGAAAGAAACAAAGACGGTTAACGATGCTCGCATTAAAGCAA
GAGGAAGGGTTACTGGGTTGGAAATCTAAGAAGCTTCACATTAGGGAACTTCATGTA
25 GCTACGGATGGTTTAGTCCAAGAGTATTCTGGTGTGGTGGGTTGGTAATGTCTAC
AGAGGAAAATTGGGGATGGACAGTGGTGCAGTGAAACGATTGAAAGATGTGAATGGA
ACCTCCGGGAACTCACAGTTCGTACTGAGCTTGAGATGATCAGCTAGCTGTTCATAGG
AATTGCTTCGGTTATCGGTTATTGTCGAGTTCTAGCGAAAGACCTTCTGTTACCCCT
TACATGTCCAATGGCAGCGTCGCCTCTAGGCTCAAAGCTAACGCCAGCGTGGACTGGAAC
30 ACAAGGAAGAAGATAGCGATTGGAGCTGCAAGAGGGTTTTTATCTACACGAGCAATGC
GATCCCAAGATTATTCACCGAGATGTCAAGGCAGCAAACATTCTCTAGATGAGTATTTT
GAAGCAGTTGGGGATTGGACTAGCAAAGCTACTCAACCACGAGGATTACATGTC
ACAACCGCGTTAGAGGAACCTGGTCACATTGCACCTGAGTATCTCCACCGGTCA
TCATCTGAGAAAACGATGTCTTGGGTTGGTATACTTTGCTAGAGCTCATCACAGGA
35 ATGAGAGCTCTGAGTTGGCAAGTCTGTTAGCCAGAAAGGAGCTATGCTAGAATGGGTG
AGGAAGCTACACAAGGAAATGAAAGTAGAGGAGCTAGTAGACCGAGAACTGGGACAACC
TACGATAGAATAGAAGTTGGAGAGATGCTACAAGTGGCACTGCTCTGCACTCAGTTCTT
CCAGCTCACAGACCCAAAATGTCAGTAGTTCACTGAGATGCTGAAGGAGATGGATTAGCT
GAGAGATGGGCTGCTTCACATGACCATTCACATTCTACCATGCCAACATGTCTTACAGG
40 ACTATTACCTCTACTGATGGCAACAAACCAACATCTGTTGGCTCCTCAGGATT

GAAGATGAAGATGATAATCAAGCGTTAGATTGCCATTGGAACTATCTGGTCCAAGG
TAGtaaatcttggacacagaaaagaaacagatataatccccatgacttcaattttgtt

5 Predicted amino acid sequence of the *Arabidopsis thaliana* RKS4 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).

10 At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 2 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate
15 bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for O-
20 glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eighth domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably
25 also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

30

MVVMKLIKMKIFSVLLLL
CFFVTCSSLSEPRNPEV

EALINIKNELHDP

35 HGVFKNWDEFNSVD

PCSWTMISCSSDNLVIGL

GAPSQSLSGTLS

G SIGNLTNLROVSLQNNNISGKI

PPEICSLPKLQTLDLSNNRFSGEI

PGSVNQLSNLQYRLRLNNNSLSGPF

5 PASLSQIPHLSFLDLSYNNLRGPV

PKFPARTFNVAGNPLICKNS

LPEICSGSISASPL

SVSLRSSSGRRTN

10

ILAVALGVSLGFAVSVIL

SLGFIWY

RKKQRRLTMLRINKQEE

15 GLLGLGNLRSFTFRELHVAT

DGFSSKSILGAGGGFGNVYRGKFGD

GTVVAVKRLKDVNNGTSGNSQFR

TELEMISLAVHRNLLRLIGYCA

20 SSSERLLVYPYMSNGSVASRLK

AKPALDWNTTRKKIAIGAA

RGLFYLHEQCDPKIIHRDVKAA

NILLDEYFEAVVGDFGLAKLLN

HEDSHVTTAVRGTVGHIAPEYL

25 STGQSSEKTDVFGFGILLLELI

TGMRALEFGKSVSQKGAMLEW

VRKLHKEMKVEELVDRELGTTY

DRIEVGEMLQVALLCTQFLPAH

RPKMSEVVQMLE

30

GDGLAERWAASHDHSHFYHANM

SYRTITSTDGNNQTKHLFG

SSGFEDEDDNQALDSFAMELSGPR

35

Arabidopsis thaliana RKS5 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

10 ctagagaattttatacttttctacg**ATGGAGATTCTTGATGAAGTTCTGTTTA**
GGAATCTGGGTTTATTATTACTCTGTTCTGCCATGGATAGTCTTTA
TCTCCCAAGGTGGCTGCGTTAATGTCAGTGAAGAACAAAGATGAAAGATGAGAAAGAGGTT
TTGTCTGGTTGGGATATTAACTCTGTTGATCCTGTACTTGGAACATGGTTGGTGTCT
TCTGAAGGTTTGTGGTTCTCTAGAGATGGCTAGTAAAGGATTATCAGGGATACTATCT
15 ACTAGTATTGGGAATTAACTCATCTCATACTTTGTTACTTCAGAAATAATCAGTTAATC
GGTCCGATTCCCTCTGAGTTAGGCCAATCTCTGAGCTTGAAACGCTTGATTTATCGGGG
AATCGGTTAGTGGTGAATCCCAGCTTCTTAGGGTCTTAACTCACTTAAACTACTTG
CGGCTTAGCAGGAATCTTATCTGGCAAGTCCTCACCTCGTCGCTGCCCTCAGGT
CTTCTTCTGGATCTATCTTCAACAACTAAGCGGACCAACTCCGAATATATCAGCA
20 AAAGATTACAGGAAATGCATTCTTGTTGGTCCAGCTCCCAAGAGCTTGCTCAGATGC
TACACCTGTGAGAAATGCTGCAATCGATCTGCAGCGACGGGTTGTCTGAAAAGGACAAT
AGCAAACATCACAGCTTAGTGCTCTTGCATTGGCATTGTTGCCTTATCATE
TCCCTAATGTTCTCTGGTCTGGCATCGATCACGTCTCAAGATCACAC
GTGCAGCAAGACTACGAATTGAAATCGGCCATCTGAAAGGTTCAAGTTTCCGAAATA
25 CAAACCGCAACAAGCAATTAGTCAAAGAACATTGGACAAGGAGGGTTGGGATG
GTTTATAAAGGGTATCTCCAAATGAAACTGTGGTGGCAGTTAAAGATTGAAAGATCCG
ATTTATACAGGAGAAGTTCAAGTCAACCGAAGTAGAGATGATTGGCTAGCTGTTCAC
CGTAACCTTACGCCCTTGGATTCTGTATGACCCCGGAAGAGAGAAATGCTGTGAT
CCGTACATGCCAAATGGAAGCGTAGCTGATCGCTGAGAGATTGGAATCGGAGGATAAGC
30 ATTGCACTCGGCGCAGCTCGAGGACTTGTACTTGCACGAGCAATGCAATCCAAAGATT
ATTCACAGAGACGTCAAAGCTGCAAATATTCTACTTGATGAGAGCTTGAAAGCAATAGTT
GGCGATTGGTCTAGCAAAGCTTTAGACCAGAGAGATTCAACATGTCACCTCCAGTC
CGAGGAACCATTGGACACATCGCTCCCGAGTACCTTCCACTGGACAGTCCTCAGAGAAA
ACCGATGTTTCCGATTGGAGTACTAATCCTGAAACTCATAACAGGTCATAAGATGATT
35 GATCAAGGCAATGGTCAAGTCGAAAAGGAATGATATTGAGCTGGTAAGGACATTGAAA
GCAGAGAAGAGATTGCGAGAGATGGTGGACAGAGATTGAAGGGAGAGTTGATGATTG
GTGTTGGAGGAAGTAGTGGATTGGCTTGCTTGTACACAGCCACATCGAACTCAAGA
CCGAGGATGTCTCAAGTGTGAAGGTAAGAGTTAGTGGAACAGTGTGAAGGAGGG
TATGAAGCTAGAGCTCAAGTGTCTAGGAACATACAGTAATGGTCATGAAGAGCAGTCC
40 TTTATTATTGAAGCCATTGAGCTCTGGACCACGATGatagacttcatagtgtcttaac

tagtcttcttgattttgttcattgtcatggc

Predicted amino acid sequence of the *Arabidopsis thaliana* RKS5 protein.

5 Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).
At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains no
10 leucine zipper motif, in contrast to the other RKS proteins. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues.
15 The fifth domain contains many serine residues, and is likely to be a site for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eighth domain represents a serine /
20 threonine protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein /
25 protein interactions.

MEISLMKFLFLGIWVYYYYS

VLDSVVSAMDSLLSPKV

30

AALMSVKNPKMKDE

KEVLSGWDINSVD

PCTWNMVGCSSSEGTVVS

35

LEMASKGLSGILS

T SIGELTHLHTLLLQNNQLTGPI

PSELGQLSELETLDLSGNRFSGEI

PASLGFLTHLNYLRLSRNLLSGQV

PHLVAGLSGLSFLDLSFNNLSGPT
PNISAK DYRKCISLWSSFPR

ALLRCYTCEKCCNR

5 SAATGLSEKDNSK

HHSLVLSFAFGIVV

AFIISLMFLFFWVLWH

10 RSRLSRSHVQQDYEF

EIGHLKRFSFREIQTAT

SNFSPKNILGQGGFGMVYKGYLPN

GTVVAVKRLKDPIYTGEVQFQ

15 TEVEMIGLAVHRNLLRLFGFCM

TPEERMLVYPYMPNGSVADRLR

DWNRRISIALGAA

RGLVYLHEQCNPKIIRDVKAA

NILLDESFEAIVGDFGLAKLLD

20 QRDSHVTTAVRGTIGHIAPEYL

STGQSSEKTDVFGFGVLILELI

TGHKMI DQGNGQVRKGMI LSW

VRTLKAEKRFAEMVDRDLKG

DDLVLEEVVELALLCTQPHPNL

25 RPRMSQVLKV

LEGLVEQCEGGYEARA

PASVSRNYSNGHEEQSFIIIEAIELSGPR

30

Arabidopsis thaliana RKS6 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

The first stopcodon has been underlined.

5 Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

attgttccttctttgggatttctcctggatggaaccagctcaattaatgagatgag

10 **ATG**AGAATGTCAGCTGCAGAAGATGGCTATGGCTTACTCTCTTGTGCTGT
TTATGCTCATTGTGTCTCCAGATGCTCAAGGGATGCAGTGGTGCAGGATCTCC
TTACGTGCATTACCGAATCAGCTAAGTGACTGGAATCAGAACCAAGTTAACCTGCAC
TGGTCCAAGTTATTGTGATGACAAAAACTTGTCACTCTTACATTGTCAGATATG
AACTCTCGGGAACCTGTCTCAAGAGTAGGAATCCTAGAAAATCTCAAGACTCTTACT
15 TTAAAGGGAAATGGAATTACGGGTGAAATACCAGAAGACTTGGAAATCTGACTAGCTTG
ACTAGTTGGATTGGAGGACAATCAGCTAAGTGGTGTACCCATCCACTATCGGTAAT
CTCAAGAAACTTCAGTTCTGACCTGAGTAGGAACAAACTTAATGGGACTATTCCGGAG
TCACTCACTGGTCTCCAAACCTGTTAACCTGCTGCTGATTCCAATAGTCTCAGTGGT
CAGATTCCCAAAGTCTGTTGAGATCCAAAATATAATTTCACGTCAAACAACTTGAAT
20 TGTCGGGGTGTCAACCTCACCCCTGTTATCCGGTTGCCCATTCAGGTGATTCAAGC
AAGCCTAAAATGGCATTATTGCTGGAGTTGGCTGGAGTTACAGTTGTTCTTTGGA
ATCTTGTTGTTCTGCAAGGATAGGCATAAAGGATATAGACGTGATGTGTTGTG
GATGTTGCAGGTGAAGTGGACAGGAGAATTGCATTGGACAGTTGAAAAGGTTGCATGG
AGAGAGCTCCAGTTAGCGACAGATAACTCAGCGAAAAGAATGTACTTGGTCAAGGAGGC
25 TTTGGAAAGTTACAAAGGAGTGCTCCGGATACACCCAAAGTTGCTGTGAAGAGATTG
ACGGATTCGAAAGTCCTGGTGGAGATGCTGCTTCCAAAGGGAAAGTAGAGATGATAAGT
GTAGCTGTTCATAGGAATCTACTCCGTCTATCGGGTCTGCACCACACAAACAGAACGC
CTTTGGTTATCCCTCATGCAGAATCTAAGTCTGCACATCGTCTGAGAGAGATCAA
GCAGGGCACCAGGTCTAGATTGGAGACGAGGAAACGGATTGCCTAGGAGCAGCGCGT
30 GGTTTGAGTATTCATGAACATTGCAATCCGAAGATCATACATCGTATGTGAAAGCA
GCTAATGTGTTACTAGATGAAGATTGAAAGCAGTGGTTGGTGAAGTGGTTAGCCAAG
CTAGTAGATGTTAGAAGGACTAATGTGACTACTCAAGTTCGAGGAACAATGGTCACATT
GCACCAAGAATATTCAACAGGGAAATCATCAGAGAGAACCGATTTTGGGGTATGGA
ATTATGCTTCTTGAGCTTGTACAGGACAACCGCAATAGACTTTCACGTTGGAGGAA
35 GAAGATGATGTCTGTTACTTGACCACGTGAAGAAACTGGAAAGAGAGAACGAGATTAGGA
GCAATCGTAGATAAGAATTGGATGGAGAGTATATAAGAAGAAGTAGAGATGATGATA
CAAGTGGCTTGCTTGTACACAAGGTTCACCAAGAGACGACCAGTGTGATGTGAAAGTT
GTGAGGATGTTAGAAGGAGAAGGGCTTGGAGAGATGGGAAGAGTGGCAAAACGTGGAA
GTCACGAGACGTCATGAGTTGAACGGTTGCAGAGGAGATTGATTGGGTGAAGATTCT
40 ATGCATAACCAAGATGCCATTGAATTATCTGGTGGAAAGATGAccaaaacatcaaacctt

Predicted amino acid sequence of the *Arabidopsis thaliana* RKS6 protein.

Different domains are spaced and shown from the N-terminus

5 towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 3 leucine residues, each

10 separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain

15 contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for O-glycosylation. The sixth domain contains a single

transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown

20 function. The eight domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions.

The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single

25 leucine rich repeat, probably involved in protein / protein interactions.

MRMFSL

QKMAMAFATLLFFACLCSFVSPDAQG

30

DALFALARISLRALP

NQLSDWNQNQVN

PCTWSQVICDDKNFVTSL

35

TLSDMNFGSTLSSRV

GILENLKTLTLKGNGITGEI

PEDFGNLTSLTSLDLEDNQLTGRI

PSTIGNLKKLQFLTLSRNKLNGTI
PESLTGLPNLLNLLDSNSLSGQI
PQSLFEIPKYNFTSNNLNCGG

5 RQPHPCVSAVAHSGDSSKPKTG

IIAGVVAGVTVVL
FGILLEFLFC

10 KDRHKGYRRDVFDVAGE
VDRRIAFGQLKRFRAWRELQLAT

DNFSEKNVLGQGGFGKVKGVLPD
TPKVAVKRLTDFESPGDAAFQ

15 REVEMISVAHRNLLRLIGEFT
TQTERLLVYPFMQNLSLAHRLR
EIKAGDPVLDWETRKRIALGAA
RGFEYLHEHCNPKIIHRDVKAA
NVLLDEDFEAVVGDFGLAKLVD
20 VRRTNVTTQVRGTMGHIAPEYL
STGKSSERTDVFGYGYGIMLLELV
TGQRAIDFSRLEEDDVLLLDH
VKKLEREKRLGAIVDKNLDGEY
IKEEVEMMIQVALLCTQGSPED
25 RPVMSEVVRMLE

GEGLAERWEEWQNVEVTRRHEFE

RLQRRFDWGEDSMHNQDAIELSGGR

Arabidopsis thaliana RKS7 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

10 acatcttgctttctgctcatccctgtttcaaca**ATGGAGAGTACTATTGTTATGATGA**
TGATGATAACAAGATCTTCTTTGCTTCTGGGATTTATGCCTCTGCTCTG
TTCACGGATTGCTTCTCCTAAAGGTGTTAAC**TTGA**GTGCAAGCTTGATGGACATAA
AAGCTTCATTACATGATCCTCATGGTGTCTGATAACTGGGATAGAGATGCTGTTGATC
CTTGTAGTTGGACAATGGTCACTTGTCTGAAA**ACTTGT**CATTGGCTTAGGCACAC
15 CAAGTCAGAATTATCTGGTACACTATCTCAAGCATTACCAACTAACAAATCTCGGA
TTGTGCTGGCAGAACAAACATAAAAGGAAAATTCTGCTGAGATTGGTCGGCTTA
CGAGGCTTGAGACTCTGATCTTCTGATAATTCTCACGGTGA**AAATT**CCTTTTCAG
TAGGCTATCTACAAAGCCTGCAATATCTGAGGCTTAACAA**ACATT**CTCTGGAGTGT
TTCCTCTGTCACTATCTAATATGACTCAACTTGCCTTCTGATTATCATACAA**ACATC**
20 TTAGGGCCTGTTCCAAGATTGCTGCAAAGACGTTAGCATCGTGGGAACCCGCTGA
TATGTCCAACGGGTACCGAACCAACAGACTGCAATGGAACAA**ACATT**GATA**ACCT**ATGTCTATGA
ACTTGAATCAA**AC**ACTGGAGTTCCCTTATACGCCGGTGGATCGAGGA**ATC**ACAAA**ATGGCAA**
TCGCTGTTGGATCCAGCGTTGGACTGTATCATTAATCTCATTGCTGTTGGTTGTT
TCTGGTGGAGACAAAGACATAACCAAAACACATTCTTGATGTTAAAGATGGGA**ATCATC**
25 ATGAGGAAGTTCACTGGAAACCTGAGGAGATTGGTTCA**GGGAGCTTCAGATTGCGA**
CCAATAACTCAGCAGTAAGAA**ACTT**TATTGGGGAAAGGTGGCTATGGA**AAATGT**TACAAAG
GAATA**ACTTGGAGA**TAGTACAGTGGTGCAGTGA**AAAGGCTTAAAGATGGGAGCATTGG**
GAGGAGAGATTCA**GGTT**CAGACAGAA**AGTGTGAAATGATCAGTTAGCTGTT**CATCGAA**AC**
TCTTAAGACTCTACGGTTCTGCATCACACAA**ACTGAGAAGCTCTAGTTATCCTTATA**
30 TGTCTAATGGAAAGCGTTGCATCTCGAATGAA**AGCAAAACCTGTTCTGACTGGAGCATAA**
GGAAGAGGATAGCCATAGGAGCTGCAAGAGGGCTTGTGTATCTCCATGAGCA**ATGTGATC**
CGAAGATTATCCACCGCGATGTCAA**AGCAGCGAATATACTTCTGATGACTACTGTGAAG**
CTGTGGTTGGCGATTGGTTAGCTAA**ACTCTGGATCATCAAGATTCTCATGTGACAA**
CCGCGGTTAGAGGCACGGTGGGTCA**CATTGCTCCAGAGTATCTCTCAACTGGTCAATCCT**
35 CTGAGAAA**ACAGATGTTTGGCTCGGGATTCTTCTTGAGCTTGTAACCGGACAAA**
GAGCTTTGAGTTGGTAAAGCGGCTAAC**CCAGAAAGGTGTGATGCTTGATTGGTTAAAA**
AGATTCAAGAGAAGAA**ACTTGAGCTACTTGTGGATAAAGAGTTGTTGAAGAAGAAGA**
GCTACGATGAGATTGAGTTAGACGAA**ATGGTAAGAGTAGCTTGTGACACACAGTACC**
TGCCAGGACATAGACCAAA**ATGTCTGAAGTTGTCGAATGCTGGAAGGAGATGGACTTG**
40 CAGAGAAA**ATGGGAAGCTTC**AAAGATCAGACAGTGTTC**AAAATGTAGCAACAGGATAA**

ATGAATTGATGTCATCTCAGACAGATACTCTGATCTTACCGATGACTCTAGTTACTTG
TGCAAGCAATGGAGCTCTGGTCCTAGATGAaatctatacatgaatctgaagaagaaga
agaacatgcacatgtttcttgaatcaagagggattcttgggggggggggggggggggggg
ttttttggagggaaatgttgtctgtactgtataggcttggtaagaagttat
5 tactgcacttagggtaattcaaaggttttacataaaaaatgattagttgcgttgaata
gagggAACACTTGGAGATTCATGTATGAAATTGGaaaaaaaaaaaaaaaaaaaaaa

10 Predicted amino acid sequence of the *Arabidopsis thaliana* RKS7 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al (1997).

15 At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 3 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate
20 bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eighth domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably
25 also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

30

35

MESTIVMMMMITRSFF
CFLGFLCLLCSSVHGLLSPKGVNFEV

QALMDIKASLHDP
HGVLDNWDRDAVD

PCSWTMVTCSSENFVIG

5

LGTPSQNLSGTL
SPSITNLTNLRIVLLQNNNIKGKI
PAEIGRLTRLETLDLSDNFFHGEI
PFSVGYLQSLQYLRLLNNNSLSGVF
10 PLSLSNMTQLAFLDLSYNMNLSPV
PRFAA KTF SIVGNPLICPT

GTEPDCNGTTLI PMSMNL
NQTGVPLYAGGSRNHKMA

15

IAVGSSVGTVSLIFIAVGLFLWW

RQRHNQNTFFDVKDGNHHE
EVSLGNLRRFGFRELQIAT

20

NNFSSKNLLGKGYYGNVYKGILGD
STVVAVKRLKDGGALGGEIQFQ
TEVEMISLAVHRNLLRLYGFCI
TQTEKLLVYPYMSNGSVA

25

SRMKAKPVLDWSIRKRIAIGAA
RGLVYLHEQCDPKIIHRDVKAA
NILLDDYCEAVVGDFGLAKLLD
HQDSHVTAVRGTVGHIAPEYL
STGQSSEKTDVFGFGILLILELV

30

TGQRAFEFGKAANQKGVMLDW
VKKIHQEKKLELLVDKELLKKSY
DEIELDEMVRVALLCTQYLPGH
RPKMSEVVVRMLE

35

GDGLAEKWEASQRSDS
VSKCSNRINELMSSS

DRYSDLTDDSSLLVQAMELSGPR

40

Arabidopsis thaliana RKS8 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

10 gtttttttttttaccctcttggaggatctggaggagaatttgcgtttttggtaa
ATGGGGAGAAAAAGTTGAAGCTTTGGTTTGCTGCTTAATCTCACTGCTTCTG
TTAACATTGTTATGGCTGCCTCTCTAACATGGAAAGGTGATGCACAGCTTGAGA
GCTAACTAGTTGATCCAAATAATGTCTGCAAAGCTGGATCCTACGCTTGTTAATCCG
TGTACTTGGTTTCACGTAACGTGTAACAACGAGAACAGTGTATAAGAGTCGATCTGGG
15 AATGCAGACTTGTCTGGTCAGTTGGTCAGCTAGGTAGCTAGCTAAGAACTTGCAGTAC
TTGGAGCTTATAGTAATAAACATAACCGGGCCGGTCCAAGCGATCTGGGAATCTGACA
AACTTAGTGAGCTTGGATCTTACTTGAAACAGCTTCACTGGTCCAATTCCAGATTCTCTA
GGAAAGCTATTCAAGCTCGTTCTCGCTCAACAATAACAGTCTCACCGGACCAATT
CCCATGTCATTGACTAATATCATGACCCCTCAAGTTGGATCTGTCGAACAACCGATTA
20 TCCGGATCTGTTCTGATAATGGTTCTCTCGCTCTCACTCCCACAGTTTGCTAAC
AACTTGGATCTATGCGGCCAGTTACTAGCCGCTTGTCTGGATCTCCCCGTTTCT
CCTCCACCACCTTTATACCACCTCCATAGTTCTACACCAGGTGGGTATAGTGCCTACT
GGAGCCATTGCGGGAGGAGTTGCTGCTGGTCTGCTTACTATTTGCTGCCCTGCTTA
GCTTTGCTTGGTGGCGTAGAAGAAAACCTCAAGAATTCTCTTGATGTTCTGCCGAA
25 GAGGACCCCTGAGGTTCACTGGGGCAGCTTAAGCGGTTCTCTACGGGAACCTCAAGTA
GCAACTGATAGCTTCAGCAACAAGAACATTGGGCCAGGGTGGGTTCGGAAAAGTCTAC
AAAGGCCGTCTGCTGATGGAACACTTGTGCACTAAACGGCTAAAGAAGAGCGAACCC
CCAGGTGGCGAGCTCCAGTTCAAGACAGAAGTGGAGATGATAAGCATGGCGTTCACAGA
AATCTCCTCAGGCTACGCGGTTCTGTATGACCCCTACCGAGAGATTGCTTGTATCCT
30 TACATGGCTAATGGAAGTGCGCTTCTGTTGAGAGAACGTCACCATCACAGTTGCCT
CTAGCCTGGTCAATAAGACAGCAAATCGCGCTAGGATCAGCGAGGGTTGTCTTATCTT
CATGATCATTGCGACCCCCAAAATTATTACCGTGATGTGAAAGCTGCTAATATTCTGTTG
GACGAGGAATTGAGGCGGTGGTAGGTGATTCGGGTTAGCTAGACTATGGACTATAAA
GATACTCATGTCACAACGGCTGTGCGTGGACTATTGGACACATTGCTCCTGAGTATCTC
35 TCAACTGGAAAATCTCAGAGAAAATGATGTTTGCTACGGGATCATGCTTGGAA
CTGATTACAGGTCAGAGAGCTTTGATCTGCAAGACTGGCGAATGACGATGACGTTATG
CTCCTAGATTGGGTGAAAGGGCTTTGAAGGAGAAGAAGCTGGAGATGCTGTGGATCCT
GACCTGCAAAGCAATTACACAGAACAGCAGAAGTAGAACAGCTCATACAAGTGGCTTCTC
TGCACACAGAGCTCACCTATGGAACGACCTAAGATGTCTGAGGTTGTCGAATGCTTGAA
40 GGTGACGGTTAGCGGAGAAATGGGACGAGTGGCAGAAAGTGGAAAGTTCTCAGGCAAGAA

GTGGAGCTCTCTCACCCACCTCTGACTGGATCCTGATTGACTGATAATCTTCAT
GCTATGGAGTTGTCTGGTCCAAGATAAacgacattgttaattgcctaacagaaaaagagaa
agaacagagaaatattaagagaatcacttctgtattctt

5

Predicted amino acid sequence of the *Arabidopsis thaliana* RKS8 protein.

Different domains are spaced and shown from the N-terminus

10 towards the C-terminus. Overall domain structure is similar as described in Schmidt *et al.* (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 4 leucine residues, each separated by seven other amino

15 acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline 20 residues, and to be a site for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine 25 protein kinase domain (Schmidt *et al.* 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

30 MGRKKFEAFGFVCLISLLLLFNSL
WLASSNMEG

DALHSILRANLVDP
NNVLQSWDPTLVN

35 PCTWFHVTCNNENSVIRV

DLGNADLSGQLV
P QLGQLKNLQYLELYSNNITGPV
40 PSDLGNLTNLVSLDLYLNSFTGPI

PDSLGKLFKLRFLRLNNNSLTGPI
PMSLTNIMTLQVLDLSNNRLSGSV
PDNGSFSLFTPISFANNLDLCGPV

5 TSRPCPGSPPFSPPPP
FIPPPIVPTPGGYSATG

AIAGGVAAGAAL
LFAAPALAFAWW

10 RRRKPQEFFFDVPAEEDPE
VHLGQLKRFSLRELQVAT

DSFSNKNILGRGGFGKVKYKGRLAD

15 GTLVAVKRLKEERTPGGELQFQ
TEVEMISMAYHRNLLRLRGFCM
TPTERLLVYPYMANGSVASCLR
ERPPSQLPLAWSIRQQIALGSA
RGLSYILHDHCDPKIIHRDVKA
20 NILLDEEFEAVVGDFGLARLMD
YKDTHVTTAVRGTIGHIAPEYL
STGKSSEKTDVFGYGYGIMLLELI
TGQRAFDLARLANDDDVMILLDW
VKGLLKEKKLEMLVDPDLQSNY
25 TEAEVEQLIQLVALLCTQSSPME
RPKMSEVVRMLE

GDGLAEKWDEWQKVEVLRQEVELS

30 SHPTSDWILDSTDNLHAMELSGPR

Arabidopsis thaliana rks10 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

10 atcagggttttaacaatgatggat~~ttt~~ctgatgagggatagtttagggttttt
taatctttaggataaa**ATGGAACGAAAGATTAATGATCCCTGCTCTTGGTTGATT**
CTCGTTGGATTGGTCTCAGAGTCTCGGGCAACGCCGAAGGTGATGCTCTAAGTGCA
CTGAAAAACAGTTAGCCGACCCATAAAGGTGCTCAAAGTTGGATGCTACTCTTGTT
ACTCCATGTACATGGTTCATGTTACTGCAATAGCGACAATAGTGTACACGTGTTGAC
15 CTTGGGAATGCAAATCTATCTGGACAGCTCGTAATGCAACTGGTCAGCTCCAAACTTG
CAGTACTTGGAGCTTATAGCAATAACATTACTGGACAATCCCAGAACAGCTGGAAAT
CTGACGGAATTGGTGAGCTGGATCTTACTTGAACAATTAAAGCAGGGCTATTCCATCA
ACTCTCGGCCGACTTAAGAAACTCCGTTCTTGCCTTAATAACAATAGCTTATCTGGA
GAAATTCCAAGGTCTTGACTGCTGCTGACGCTACAAGTTCTGGATCTCTCAAACAAT
20 CCTCTACCGGAGATATTCTGTTAATGGTCCTTCACTTTCACTCCAATCAGTTT
GCCAACACCAAGTTGACTCCCCTCCTGCATCTCCACCGCCTCTATCTCCTACACCG
CCATCACCTGCAGGGAGTAATAGAATTACTGGAGCGATTGCGGGAGGAGTTGCTGCAGGT
GCTGCACTTCTATTGCTGTTCCGGCATTGCACTAGCTTGGTGGCGAAGGAAAAGCCG
CAGGACCACTTCTTGATGTACCAGCTGAAGAGGACCCAGAAGTTCATTTAGGACAATG
25 AAGAGGTTTCATTGCGTGAACATACAAGTTGCTCGGATAATTAGCAACAAGAACATA
TTGGGTAGAGGTGGTTTGGTAAAGTTATAAAGGACGGTTAGCTGATGGTACTTTAGTG
GCCGTTAAAAGGCTAAAAGAGGAGCGCACCCAAAGGTGGCGACTGCAGTTCCAGACAGAG
GTTGAGATGATTAGTATGGCGGTTACAGAAACTTGCTCGGCTTGTGGATTTCATG
ACTCCAACCGAAAGATTGCTTGTATCCCTACATGGCTAATGGAAGTGTGCCTCCTGT
30 TTAAGAGAACGTCGGAGTCCCAGCCACCCTGATTGGCAAAGAGACAGCGTATTGCG
TTGGGATCTGCAAGAGGGCTTGCATTTACATGATCATTGCGACCCAAAGATTATTGAT
CGAGATGTGAAAGCTGCAAATATTGTTGGATGAAGAGTTGAAGCCGTGGTTGGGAT
TTTGGACTTGCAAAACTCATGGACTACAAAGACACACATGTGACAACCGCAGTGCCTGG
ACAATTGGTCATATAGCCCTGAGTACCTTCCACTGGAAAATCATCAGAGAAAACCGAT
35 GTCTTGGGTATGGAGTCATGCTTCTGAGCTTATCACTGGACAAAGGGCTTGTGATCTT
GCTCGCCCTCGCGAATGATGATGATGTCATGTTACTAGACTGGGTGAAAGGGTTGTTAAA
GAGAAGAAAATTGGAAGCAGTAGTAGATGTTGATCTCAGGGTAATTACAAAGACGAAGAA
GTGGAGCAGCTAACCAAGTGGCTTACTCTGCACTCAGAGTTACCAATGGAAAGACCC
AAAATGTCTGAAGTTGTAAGAATGCTGAAGGAGATGGTTAGCTGAGAGATGGAAAGAG
40 TGGCAAAAGGAGGAAATGTTCAGACAAGATTCAACTACCCAACCCACCATCCAGCCGTG

TCTGGCTGGATCATTGGCGATTCCACTTCCCAGATCGAAAACGAATACCCCTGGGTCCA
AGATAAgattcgaaacacgaatgttttctgtattttctgtatttattgag
ggtttttagcttc

5

Predicted amino acid sequence of the *Arabidopsis thaliana* RKS10 protein.

Different domains are spaced and shown from the N-terminus

10 towards the C-terminus. Overall domain structure is similar as described in Schmidt *et al.* (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 4 leucine residues, each separated by seven other amino

15 acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline 20 residues, and to be a site for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eight domain represents a serine / threonine 25 protein kinase domain (Schmidt *et al.* 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

30 MERRLMIPCFFWLILVL
DLVLRVSGNAEG

DALSALKNSLADP
NKVLQSWDATLVT

35 PCTWFHVTCTNSDNSVTRV

DLGNANLSGQLV
M QLGQLPNLQYLELYSNNITGTI
40 PEQLGNLTELVSLDLYLNNLSGPI

PSTLGLRKLRFLRLNNNSLSGEI
PRSLTAVLTLQVLDSLNNPLTGDI
PVNGSFSLTPISFANTK LT PL

5 PASPPPPISPTPPSPAGSNRITG

AIAGGVAAGAAL
LFAVPAIALAWW

10 RRKKPQDHFFDVPAEEDPE

VHLGQLKRFSLRELQVAS

DNFSNKNILGRGGFGKVKYKGRLAD
GTLVAVKRLKEERTQGGELQFQ

15 TEVEMISMAVRNLLRLRGFCM

PTERLLVYPYMANGSVASCLR
ERPESQPPLDWPKRQRIALGSA
RGLAYLHDHCDPKIIHRDVKAA
NILLDEEFEAVVGDFGLAKLMD

20 YKDTHVTTAVRGTTIGHIAPEYL

STGKSSEKTDVFGYGVMLLELI
TGQRAFDLARLANDDDVMLLDW
VKGLLKEKKLEALVDVDLQGNY
KDEEVEQLIQVALLCTQSSPME

25 RPKMSEVVRMLE

GDGLAERWEWQKEEMFRQDFNYPTH

PAVSGWIIGDSTSQIENEYPSGPR

Arabidopsis thaliana RKS 11 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

10 ttgttaacctctcgtaactaaaatcttcc**ATGGTAGTAGTAACAAAGAAGACCATGAAGA**
TTCAAATTCATCTCCTTACTCGTTCTGTCCTCTGTTCTACTCTCACTCTATCTT
CTGAGCCCAGAAACCTGAAGTTGAGGCAGTGAAGTATAAGGAACAATTGCATGATC
CTCATGGAGCTTGAACAATTGGGACGAGTTTCAGTTGATCCTGTAGCTGGCTATGA
TCACTTGCTCTCCGACAACCTCGTATTGGACTAGGAGCGCCGAGCCAGTCTCTCGG
15 GAGGTTTATCTGAGTCTATCGGAAATCTCACAAATCTCCGACAAGTGTCAATTGCAAAATA
ACAACATCTCCGGAAAATTCCACCGGAGCTCGGTTTCTACCCAAATTACAAACCTTGG
ATCTTCCAACAACCGATTCTCCGGTGACATCCCTGTTCCATCGACCAGCTAACGCAGCC
TTCAATATCTGAGACTCAACAACACTTTGTCTGGGCCCTCCCTGCTTCTTGTCCC
AAATTCCCTCACCTCTCCTTCTGGACTTGTCTACAACAATCTCAGTGGCCCTGTTCTA
20 AATTCCCAGCAAGGACTTTAACGTTGCTGGTAATCCTTGATTGTAGAAGCAACCCAC
CTGAGATTGTTCTGGATCAATCAATGCAAGTCCACTTCTGTTCTTGAGCTTTCAT
CAGGACGCAGGTCTAACAGATTGGCAATAGCTCTAGTGTAAAGCCTGGCTCTGTTGTTA
TACTAGTCCTGCTCTGGGCCTTTGTTGGTACCGAAAGAAACAAAGAAGGCTACTGA
TCCTTAACCTAACGCAGATAAACAAAGAGGAAGGGCTCAAGGACTTGGGAATCTAAGAA
25 GCTTCACATTCAAGAGAACTCCATGTTATACAGATGGTTCAAGAACATTCTCG
GCGCTGGTGGATTCGGAATGTGTACAGAGGCAAGCTGGAGATGGGACAATGGTGGCAG
TGAAACGGTTGAAGGATATTAATGGAACCTCAGGGGATTACAGTTCTGTTGAGCTAG
AGATGATTAGCTTAGCTGTTCATAAAGAATCTGTTGCGTAATTGGTTATTGCGCAACTT
CTGGTGAAGGCTTCTGTTACCTTACATGCCATGGAAGCGTGCCTCTAACGCTTA
30 AATCTAAACCGGCATTGGACTGGAACATGAGGAAGAGGATAGCAATTGGTGCAGCGAGAG
GTTTGGTGTATCTACATGAGCAATGTGATCCAAAGATCATTGATAGAGATGTAAAGGCA
CTAATATTCTCTTAGACGAGTGCTTGAAGCTGTTGGTGAATTGGACTCGCAAAGC
TCCTTAACCATGCGGATTCTCATGTCACAACGCGGTCCGTGGTACGGTTGGCCACATTG
CACCTGAATATCTCTTACGGTCAGTCTGAGAAAACCGATGTGTTGGGTTGGTA
35 TACTATTGCTCGAGCTCATAACCGGACTGAGAGCTCTGAGTTGGTAAAACCGTTAGCC
AGAAAGGAGCTATGCTGAATGGGTGAGGAATTACATGAAGAGATGAAAGTAGAGGAAC
TATTGGATCGAGAACTCGGAACAACTACGATAAGATTGAAGTTGGAGAGATGTTGCAAG
TGGCTTGCTATGCACACAAATCTGCCAGCTCATCGTCCTAAATGTCTGAAGTTGTT
TGATGCTTGAAGGCAGTGGATTAGCCGAGAGATGGGCTGCTCGCATAACCATTACATT
40 TCTACCATGCCAATATCTCTTCAAGACAATCTCTGTCTACTACTTCTGTCTCAA

GGCTTGACGCACATTGCAATGATCCAACTTATCAAATGTTGGATCTTCGGCTTCGATG
ATGACGATGATCATCAGCCTTAGATTCCCTTGCCATGGAACTATCCGGTCCAAGATAAC
acaatgaaagaaaatcattttacgatggatcaaacaatccaatgaaaaaa

5

Predicted amino acid sequence of the *Arabidopsis thaliana* RKS11 protein.

10 Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt et al. (1997).
At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a
15 leucine zipper motif, containing 3 leucine residues, each separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each
20 approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for O-glycosylation. The sixth domain contains a single transmembrane domain after which the predicted intracellular
25 domains are positioned. The seventh domain has an unknown function. The eighth domain represents a serine / threonine protein kinase domain (Schmidt et al. 1997) and is probably also containing sequences for protein / protein interactions. The ninth domain has an unknown function. The last and tenth
30 domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein interactions.

MVVVTKKTMKIQIHL^LYSFLFL

35 CFSTLTLSS^EPRNPEV

EALISIRNNNLHDP

HGALNNWDEF^SV^D

PCSWAMITCSPDNLVIGL

GAPSQSLSGGLS

5 ESIGNLTNLRQVSLQNNNISGKI
PPELGEFLPKLQTLDSLNNRFSGDI
PVSIDQQLSQQYLRLNNNSLSGPF
PASLSQIPHLSFLDSYNNLSGPV
PKFPARTFNVAGNPLICRSN

10 PPEICSGSINASPL
SVSLSSSSGRRSNR

LAIALSVSLGSVVIL

15 VLALGSFCWY

RKKQRRLILNLNGADKQEE
GLQGLGNLRSFTFRELHVYT

20 DGFSSKNILGAGGFGNVYRGKLD
GTMVAVKRLKDINGTSGDSQFR
MELEMISLAVHKNLLRLIGYCA
TSGERLLVYPYMPNGSVASKLK
SKPALDWNRKRIAIGAA

25 RGLYLHEQCDPKIIHRDVKAA
NILLDECFEAVVGDFGLAKLLN
HADSHVTTAVRGTVGHIAPEYL
STGQSSEKTDVFGFGILLLELI
TGLRALEFGKTVSQKGAMLEW

30 VRKLHEEMKVEELLDRELGTNY
DKIEVGEMLQVALLCTQYLPAH
RPKMSEVVLMLE

35 GDGLAERWAASHNHSHFYHANI
SFKTISSLSTTSVSRLDACNDPTYQMFG

SSAFDDDDHQPLDSFAMELSGPR

Arabidopsis thaliana RKS12 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

10 tttaaaaacccgttagttctcaattctcatgactttgcttttagtcttagaagtggaaa
ATGGAACATGGATCATCCCGTGGCTTATTGGCTGATTCTATTCTCGATTTGTTCC
AGAGTCACCGGAAAAACACAAGTTGATGCTCTATTGCTCTAAGAACAGCTTATCATCA
GGTGACCATAACAAACAATATACTCCAAAGCTGGAATGCCACTCACGTTACTCCATGTTCA
TGGTTCATGTTACTTGCAACTGAAAACAGTGTACTCGTCTGACCTGGGGAGTGCT
15 AATCTATCTGGAGAACTGGTGCCACAGCTGCTCAGCTTCAAATTGCACTTGAA
CTTTTTAACAAATAATATTACTGGGGAGATACTGAGGAGCTGGCGACTTGATGGAACTA
GTAAGCTTGGACCTTTGCAAACAAACATAAGCGGTCCCACCCCTCCCTCTGGCAA
CTAGGAAAACCTCGCTTCTGCGTCTTATAACAAACAGCTTATCTGGAGAAATTCCAAGG
TCTTGACTGCTCTGCCGCTGGATGTTCTGATATCTCAAACAATCGGCTCAGTGGAGAT
20 ATTCCGTAAATGGTCCTTCGCAGTTCACTTCTATGAGTTTGCCAATAATAAATTAA
AGGCCGCGACCTGCATCTCCTTCACCATCACCTCAGGAACGTCTGCAGCAATAGTAGTG
GGAGTTGCTGCGGGTGCAGCACTTCTATTGCGCTTGCTGGTGGCTGAGAAGAAAAGTG
CAGGGTCACTTCTTGATGTACCTGCTGAAGAAGACCCAGAGGTTATTAGGACAATT
AAAAGGTTCTCCTTGCCTGAAGGCAACTGCTAGTTGCTACAGAGAAATTAGCAAAGAAATGTA
25 TTGGGCAAAGGACGTTGGTATATTGTATAAAGGACGTTAGCTGATGACACTCTAGTG
GCTGTGAAACGGCTAAATGAAGAACGTACCAAGGGTGGGAACCTGAGTTCAAACCGAA
GTTGAGATGATCAGTATGCCGTTCATAGGAACCTGCTTCGGCTCGTGGCTTGCATG
ACTCCAACGTAAAGATTACTGTTATCCCTACATGGCTAATGGAAGTGTGCTTCTTG
TTAAGAGAGCGCTCTGAAGGCAATCCAGGCCCTGACTGCCAAAAAGAACATATTGCT
30 CTGGGATCAGCAAGGGGCTCGATATTACACGATCATTGCGACCAAAAGATCATTCA
CTGGATGTGAAAGCTGCAAATATACTGTTAGATGAAGAGTTGAAGCTGTTGGAGAT
TTGGGCTAGAAAATTAAATGAATTATAACGACTCCATGTGACAACGTGCTGTACGGGGT
ACGATTGCCATATAGGCCCGAGTACCTCTCGACAGGAAAATCTCTGAGAACAGACTGAT
GTTTTGGGTACGGGGTACGTTCTGAGCTCATCACTGGACAAAAGGCTTCGATCTT
35 GCTCGGCTGCAAATGATGATGATCATGTTACTCGACTGGGTGAAAGAGGTTTGAAA
GAGAAGAAGTTGGAAAGCCTGTGGATGCAGAACTCGAAGGAAAGTACGTGGAAACAGAA
GTGGGAGCAGCTGATACAAATGGCTCTGCTCTGCACTCAAAGTTCTGCAATGGAACGTCCA
AAGATGTCAGAAGTAGTGAGAATGCTGGAAGGAGATGGTTAGCTGAGAGATGGGAAGAA
TGGCAAAAGGAGGAGATGCCAATACATGATTTAACTATCAAGCCTATCCTCATGCTGGC
40 ACTGACTGGCTCATCCCCATTCCAATTCCCTATCGAAAACGATTACCCCTGGGGCCA

AGATAActttttagaaagggtcattcttgcgggtcttcaacaagtatataataggta
gtgaagttgtaaaagcaaaacccacattcaccttgaatatcaactactataa

5

Predicted amino acid sequence of the *Arabidopsis thaliana* RKS12 protein.

Different domains are spaced and shown from the N-terminus

10 towards the C-terminus. Overall domain structure is similar as described in Schmidt *et al.* (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 2 leucine residues, each

15 separated by seven other amino acids. The third domain contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain

20 contains many serine and proline residues, and is likely to contain hydroxy-proline residues, and to be a site for O-glycosylation. The sixth domain contains a single

transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown

25 function. The eight domain represents a serine / threonine protein kinase domain (Schmidt *et al.* 1997) and is probably also containing sequences for protein / protein interactions.

The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single

30 leucine rich repeat, probably involved in protein / protein interactions.

MEHGSSRGFI

WLILFLDFVSRVTGKTQV

35

DALIALRSSLSSGDHTNNILQ

SWNATHVT

PCSWFHVTCNTENSVTRL

DLGSANLSGELV

P QLAQLPNLQYLELFNNNITGEI

5 PEELGDLMELVSLDLFANNISGPI

PSSLGKLGKLRFLRLYNNLSGEI

PRSLTALP LDVLDISNNRLSGDI

PVNGSFSQFTSMRFA NNKLRPR

10 PASPSPSPSGGTS

AAIVVGVAAGAALLFALAWWL

RRKLQGHFLDVPAAEEDPE

15 VYLGQFKRFSLRELLVAT

EKFSKRNVLGKGRFGILYKGRLAD

DTLVAVKRLNEERTKGGELOFQ

TEVEMISMAVRNLLRLRGFCM

20 TPTERLLVYPYMANGSVASCLR

ERPEGNPALDWPKRKHIALGSA

RGLAYLHDHCQKIIHLDVKAA

NILLDEEFEAVVGDFGLAKLMN

YNDSHVTAVRGTTIGHIAPEYL

25 STGKSSEKTDVFGYGVMLLELI

TGQKAFDLARLANDDDIMLLDW

VKEVLKEKKLESLVDAELEGKY

VETEVEQLIQMALLCTQSSAME

RPKMSEVVVRMLE

30 GDGLAERWEWQKEEMPIHDFNYQAY

PHAGTDWLIPYSNSLIENDYPSGPR

35

Arabidopsis thaliana RKS13 cDNA

The start codons encoding predicted the methionine residue of the gene product has been indicated by bold capitals. The first stopcodon has been underlined.

5 Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

taataaacctctaataataatggcttgctttactctgatgacaagttcaaaa**ATGGAA**

10 CAAAGATCACTCCTTGCTTCCTTATCTGCTCCTACTATTCAATTCACTCTCAGAGTC
GCTGGAAACGCTGAAGGTGATGCTTGACTCAGCTGAAAAACAGTTGTCATCAGGTGAC
CCTGCAAACAATGTTACTCAGCTGGGATGCTACTCTGTTACTCCATGTTACTGGTTT
CATGTTACTTGCAATCCTGAGAATAAAGTTACTCGTGTGACCTGGGAATGCAAAACTA
TCTGGAAAGTTGGTCCAGAACTTGGTCAGCTTTAAACTTGCAGTACTTGGAGCTTAT
15 AGCAATAACATTACAGGGGAGATACTGAGGGAGCTTGGCGACTTGGTGGAACTAGTAAGC
TTGGATCTTACGCAAACAGCATAAGCGGTCCCATCCCTCGTCTCTGGCAAACATAGGA
AAACTCCGGTTCTTGCCTTAACAAACAATAGCTTACAGGGGAAATTCCAATGACTTTG
ACTTCTGTGCAGCTGCAAGTTCTGGATATCTCAAACAATCGGTCAGTGGAGATATTCC
GTTAATGGTTCTTTTGCCTTCACCTATCAGTTTGCAGATAATAGCTTAACGGAT
20 CTTCCCGAACCTCCGCCTACTTCTACCTCTCAGGCCACCACCTCAGGGGGCAA
ATGACTGCAGCAATAGCAGGGGAGTTGCTGCAGGTGCAGCAGCTCTATTGCTGTTCCA
GCCATTGCGTTGCTTGGCTCAGAAGAAAACCACAGGACCACTTTTGATGTACCT
GCTGAAGAAGACCCAGAGGTTCAATTAGGACAACCTCAAAGGTTACCTTGCAGTACTG
TTAGTTGCTACTGATAACTTAGCAATAAAATGTATTGGGTAGAGGTGGTTGGTAAA
25 GTGTATAAAGGACGTTAGCCGATGGCAATCTAGTGGCTGTAAAAGGCTAAAAGAAGAA
CGTACCAAGGGTGGGAACCTGCAGTTCAAACCGAAGTTGAGATGATCAGTATGGCGTT
CATAGGAACCTGCTTCGGCTTCGTGGCTTGCATGACTCCAACGTAAAGATTACTTGT
TATCCCTACATGGCTAATGGAAGTGTGCTTGTAAAGAGAGCGTCCTGAAGGCAAT
CCAGCACTTGATTGGCAAAAAGAAAGCATATTGCTCTGGATCAGCAAGGGGCTTGC
30 TATTTACATGATCATTGCGACCAAAAAATCATTACCGGGATGTTAAAGCTGCTAATATA
TTGTTAGATGAAGAGTTGAAGCTGTTGGAGATTTGGGCTCGAAAATTATGAAT
TATAATGACTCCCCTGTGACAACCTGCTGTACCGGTACAATTGGCCATATAGCGCCGAG
TACCTCTGACAGGAAAATCTCTGAGAAGACTGATGTTGGGTACGGGTATGCTT
CTCGAGCTCATCACTGGACAAAAGGCTTCGATCTGCTCGGCTTGCAAATGATGATGAT
35 ATCATGTTACTCGACTGGGTGAAAGAGGTTTGAAAGAGAAGAAGTGGAAAGCCTTGTG
GATGCAGAACTCGAAGGAAAGTACGTGGAAACAGAAGTGGAGCAGCTGATAACAAATGGCT
CTGCTCTGCACTCAAAGTTCTGCAATGGAACGTCCAAAGATGTCAGAAGTAGTGAGAATG
CTGGAAGGAGATGGTTAGCTGAGAGATGGAAAGAATGGCAAAAGGAGGAGATGCCAATA
CATGATTAACTATCAAGCCTATCCTCATGCTGGCACTGACTGGCTCATCCCTATTCC
40 AATTCCCTATCGAAAACGATTACCCCTCGGGTCCAAGATAActtttagaaagggtctt

ttcttggtggttcttcaacaagtatataatagattggtaagtttaagatgcaaaaaaa
aa

5

Predicted amino acid sequence of the *Arabidopsis thaliana* RKS13 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt *et al.* (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains leucine zipper motifs, containing 2 times 2 leucine residues, each separated by seven other amino acids. The third domain

15 contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain

contains many serine and proline residues, and is likely to 20 contain hydroxy-proline residues, and to be a site for O-glycosylation. The sixth domain contains a single

transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eighth domain represents a serine / threonine

25 protein kinase domain (Schmidt *et al.* 1997) and is probably also containing sequences for protein / protein interactions.

The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein

30 interactions.

MEQRSLLCFLYLL
LLFNFTLKVAGNAEG

35 DALTQLKNSLSSGDP
ANNVILQSWDATLVT

DLGNAKLSGKLV
P ELGQLLNQYLELYSNNITGEI
PEELGDLVELVSLDLYANSISGPI
5 PSSLGKLGKLRFLRLNNNSLSGEI
PMTLTTSVQLQV LDISNNRLSGDI
PVNGSFSLFTPISFANNSLTDLPE

PPPTSTSPTPPPSG

10

GQMTAAIAAGGVAAGAAL
LFAVPAIAFAWWL

RRKPQDHFFDVPGAEEDPE

15

VHLGQLKRFTLRELLVAT

DNFSNKNVLGRGGFGKVKYKGRLAD
GNLVAVKRLKEERTKGGELOQFQ
TEVEMISMAVRNLLRLRGFCM

20

TPTERLLVYPYMANGSVASCLR
ERPEGNPALDWPKRKHIALGSA
RGLAYLHDHCDQKIIHRDVKAA
NILLDEEFEAVVGDFGLAKLMN
YNDSHVTTAVRGTIIGHIAPEYL
25 STGKSSEKTDVFGYGVMLLELI
TGQKAFDLARLANDDDIMLDW
VKEVLKEKKLESLVDALEGKY
VETEVEQLIQMALLCTQSSAME
RPKMSEVVRMLE

30

GDGLAERWEEWQKEEMPIHDFNYQA
YPHAGTDWLIPYSNSLIENDYPSGPR

35

Arabidopsis thaliana RKS14 cDNA

The start codon encoding the first predicted methionine residue of the gene product has been indicated by bold capitals.

5 The first stopcodon has been underlined.

Nucleotides predicted to encode protein sequences are in capitals. Leader and trailer sequences are in lowercase letters.

10 ctgcaccc~~ttagagattaatactctcaagaaaaacaagttt~~gattcg~~gacaaag~~**ATGTTG**
CAAGGAAGAAGAGAAGCAAAAAGAGTTATGCTTGTTC~~TCAACTTCTTCTTC~~
TTTATCTGTTCTTCTTCTGCAGAACTCACAGACAAAGTTGTTGC~~CTTAATA~~
GGAATCAAAGCTCACTGACTGATCCTCATGGAGTTCTAATGAATTGGGATGACACAGCA
GTTGATCCATGTAGCTGGAACATGATCACTGTTCTGATGGTTTCATAAGGCTAGAA
15 GCTCCAAGCCAAA~~ACTTATCAGGA~~ACTCTTCATCAAGTATTGAAATTAAACAAATCTT
CAA~~ACTGT~~TATACAGGTTATTGCAGAACAA~~TACATAACAGGAAACATCC~~CTCATGAGATT
GGGAAATTGATGAA~~ACTCAAAACACTT~~GATCTCTACCAATAACTC~~ACTGGTCAAATC~~
CCATTCACTCTTCTTACTCCAAA~~ATCTCACAGGAGGGTTAATAATAACAGC~~CTGACA
GGAACAATTCTAGCTCATTGGCAACATGACCCAACTCACTTTTG~~GATTGTCGTAT~~
20 AATAAC~~TTGAGTGGACCAGT~~CCAAAGATCACTGCCAAACATTCAATGTTATGGCAAT
TCTCAGATTG~~TCCAACAGGA~~ACTGAGAAAGACTGTAATGGGACTCAGCCTAAGCCAATG
TCAATCACCTTGAACAGT~~CTCTCAAAGAA~~ACTAAAAACCGGAAATCGCGTAGTCTCGGT
GTAAGCTTGACATGTGTTGCTTGTGATCATTGGCTTGGTTCTTGGTGGAGA
AGAACACATAACAAACAAGTATTATTCTTGACATTAATGAGC~~AAAACAAGGAAGAA~~ATG
25 TGTCTAGGG~~AACTCAAGGAGGTTA~~ATTCAAAGAACTTC~~AACTCCGCAACTAGTA~~ACTTC
AGCAGCAAGAATCTGGTC~~GGAAAAGGAGGGTTG~~AAATGTGTATAAAGGTTGTCTCAT
GATGGAAAGTATCATCGCGGTGAAGAGATTAAAGGATATAAAC~~ATGGTGGAGAGGTT~~
CAGTT~~CAGACAGAGCTG~~AAATGATAAGC~~CTTGCCTCACCGGA~~ATCTCCCGCTTA
TACGGTTCTG~~TACTACTTC~~CTGAACGGCTCTCGTTATCCTTACATGT~~CCAATGGC~~
30 AGTGT~~CGCTTCTCGT~~CTCAAAGCTAAACCGGTATTGGATTGGG~~GCACAAGAAAGCGA~~ATA
GCATTAGGAGCAGGAAGAGGGTTGCTGTATTG~~CATGAGCA~~ATGTGAT~~CCAAAGATC~~ATT
CACCGTGATGTCAAAGCTGCGAACATACTTCTGACGATTACTTGAAGCTGTTGCGGA
GATT~~TGGGTTGGCTAAGCTTGG~~ATCATGAGGAGTCGCATGTGACAACCGCCGTGAGA
GGAACAGTGGGT~~CACATTGCACCTGAGT~~ATCTCTAACAGGACAATCTCTGAGAACACA
35 GATGTGTT~~CGGTTTCGGGATTCTTCTCGA~~ATTGATTACTGGATTGAGAGCT~~CTTGAA~~
TTCGGAAAAGCAGCAAACCAAAGAGGGAGCGATACTTGATTGGTAAAGAA~~ACTACAACAA~~
GAGAACAGCTAGAACAGATAGTAGACAAGGATTGAGAGCAACTACGATAGAACAGA
GTGGAAAGAA~~ATGGTCAAGTGG~~TTGCTTGTACACAGTATCTCCCATT~~CACCGTC~~CT
AAGATGTCTGAAGTTGTGAGAACATGCTGAAGGC~~GATGGTCTGTTGAGAA~~ATGGGAGCT
40 TCTTCTCAGAGAGCAGAAACCAATAGAAC~~TTACAGTAAAC~~CTAACGAGTTTCTCCTCT

GAACGTTATCGGATCTTACAGATGATTCTCGGTGCTGGTTCAAGCCATGGAGTTATCA
GGTCCAAGATGacaagagaaaactatatgaatggcttgggtttgtaaaaaa

5

Predicted amino acid sequence of the *Arabidopsis thaliana* RKS14 protein.

Different domains are spaced and shown from the N-terminus towards the C-terminus. Overall domain structure is similar as described in Schmidt *et al.* (1997).

At the predicted extracellular domain the first domain represents a signal sequence. The second domain contains a leucine zipper motif, containing 3 leucine residues, each separated by seven other amino acids. The third domain

15 contains conserved cysteine residues, involved in disulphate bridge formation. The fourth domain contains a leucine rich repeat domain, consisting of 5 complete repeats of each approximately 24 amino acid residues. The fifth domain contains many serine and proline residues, and is likely to

20 contain hydroxy-proline residues, and to be a site for O-glycosylation. The sixth domain contains a single

transmembrane domain after which the predicted intracellular domains are positioned. The seventh domain has an unknown function. The eighth domain represents a serine / threonine

25 protein kinase domain (Schmidt *et al.* 1997) and is probably also containing sequences for protein / protein interactions.

The ninth domain has an unknown function. The last and tenth domain at the C-terminal end represents part of a single leucine rich repeat, probably involved in protein / protein

30 interactions.

MLQGRREAKKSYALFSSTFF
FFFICFLSSSSAELTDKV

35 VALIGIKSSLTDP
HGVLMNWDDTAVD

PCSWNMITCSDGFVIR

LEAPSQNLSGTLSS
SIGNLTNLQTVYRLLQNNYITGNI
PHEIGKLMKLKTLDLSTNNFTGQI
5 PFTLSYSKNLHRRV NNNSLTGTI
PSSLANMTQLTFDLDSYNNLSGPV
PRSLAKTFNVMGNSQICPT

GTEKDCNGTQPKPMSITLNSSQR
10 TKNRK

IAVVFGVSLTCVCLLIIGFGFLLWW

RRRHNKQVLFFDINEQNKE
15 EMCLGNLRRFNFKELQSAT

SNFSSKNLVGKGGFGNVYKGCLHD
GSIIIAVKRLKDIINNGGGEVQFO
TELEMISLAVHRNLLRLYGFCT
20 TSSERLLVYPYMSNGSVA
SRLKAKPVLDWGTRKRIALGAG
RGLLYLHEQCDPKIIHRDVKAA
NILLDDYFEAVVGDFGLAKLLD
HEESHVTTAVRGTVGHIAPEYL
25 STGQSSEKTDVFGFGILLLELI
TGLRALEFGKAANQRGAILDW
VKKLQQEKKLEQIVDKDLKSNY
DRIEVEEMVQVALLCTQYLPiH
RPKMSEVVRMLE

30 GDGLVEKWEASSQRAET
NRSYSKPNEFSSS

ERYSDLTDDSSVLVQAMELSGPR

35

Legends

Figure 1

5

The different domains of the predicted RKS gene product have the following functions:

The first domain of the predicted protein structure at the N-terminal end consists of a signal sequence, involved in

10 targeting the protein towards the plasma membrane. Protein cleavage removes this sequence from the final mature protein product (Jain et al. 1994, J. Biol. Chemistry 269: 16306-16310). The second domain consists of different numbers of leucine zipper motifs, and is likely to be involved in protein 15 protein dimerization. The next domain contains a conserved pair of cystein residues, involved in disulphate bridge formation. The next domain consists of 5 (or in the case of RKS3 only 4) leucine rich repeats (LRRs) shown in a gray colour, likely to be involved in ligand binding (Kobe and 20 Deisenhofer 1994, TIBS 19: 415-420). This domain is again bordered by a domain containing a conserved pair of cystein residues involved in disulphate bridge formation often followed by a serine / proline rich region. The next domain 25 displays all the characteristics of a single transmembrane domain (<http://genome.cbs.dtu.dk/services/TMHMM/>). At the predicted cytoplasmic site of protein a domain is situated with unknown function, followed by a domain with serine /threonine kinase activity (Schmidt et al. 1997, Development 124: 2049-2062). The kinase domain is followed by a domain 30 with unknown function whereas at the C-terminal end of the protein part of a leucine rich repeat is positioned, probably involved in protein-protein interactions.

Figure 2

35 Alagnment of the predicted protein sequences of the different RKS gene products from *Arabidopsis thaliana* with alignX, Vector NTI Suite 5.5 resulted in a phylogenetic tree in which

the relative homology between the different RKS members is shown.

Figure 3

5 Intron-Exon bounderries of the genomic regions on the chromosomes of *Arabidopsis thaliana* encoding the different RKS gene products. Exons are shown as boxes, whereas intron sequences are shown as lines. Sequences encoding LRR domains are displayed in gray colour, transmembrane regions in black.

10

Figure 4.

Cromosomal location of RKS genes in *Arabidopsis thaliana*, showing colocalisation with GASA genes.

15 Figure 5. A signaling complex comprising molecules of RKS proteins, ELS proteins, NDR/NHL proteins and SBP/SPL proteins.

Figure 6.

Second generation (T2) tobacco seedlings germinated on MS 20 medium. Transformations were performed with DNA clone 2212-15, representing the overexpression construct GT-RKS4-s. T2 seedlings derived from T1 plant 15.7 shows co-suppression effects while T1 plant 15.6 shows no obvious changes in level of RKS4. T1 plants 15.9 and 15.3 show overexpression effects. 25 Plant 15.7 has the lowest remaining level of RKS4 gene product, whereas plant 15.3 has the highest level of RKS4 gene product.

Figure 7

30 Second generation (T2) tobacco plants. In the upper row the offspring from a co-suppressing T1 plant 15.7 is shown. The middle row shows plants derived from a transgenic T1 plant 15.6 with no clear changes in level of RKS4 is shown while the bottom row shows plants derived from a T1 plant 15.3 in which 35 the levels of RKS4 are increased by the introduction of the overexpression construct GT-RKS4-s.

Figure 8

Second generation (T2) tobacco plants. Plants derived from a co-suppressing T1 plant 15.7 show a reduction in plant size and a delay in the initiation and outgrowth of primordia. The 5 control empty vector transgenic plants show no visible differences in growth compared with the offspring from the transgenic 15.6 plant, in which the endogenous level of RKS4 gene product was not changed. In the overexpressing plants 15.9 and 15.3 organ size was increased, similar as the number 10 of initiated leaf primordia.

Figure 9

Arabidopsis thaliana WS plants in which the endogenous level of RKS4 gene product is decreased (right picture) due to the 15 presence of a transgenic RKS4 antisense construct (GT-RKS4-16a). The left picture shows a wildtype plant of the same age as the transgenic antisense plant, grown under similar growth conditions. Plant size, organ size and number of organ primordia is decreased in the transgenic antisense plant 20 compared with the wildtype control.

Figure 10

Arabidopsis thaliana WS plants in which the endogenous level of RKS4 gene product is decreased (bottom left picture) due to 25 the presence of a transgenic RKS4 antisense construct (GT-RKS4-16a). The upper right picture shows a wildtype flower of the same age as the transgenic antisense flower, grown under similar growth conditions. Total flower size is only slightly decreased in the transgenic antisense flower compared with the 30 control flower, whereas organ size of petals is strongly decreased.

Arabidopsis thaliana WS plants in which the endogenous level of RKS4 gene product is increased (upper left picture) due to the presence of a transgenic RKS4 overexpressing construct 35 (GT-RKS4-6s). Compared with the wildtype control flower, total flower size of the transgenic flower is clearly increased. Both sepal and petal organ size is clearly increased compared

with the control.

For comparison an *Arabidopsis thaliana* WS plant is shown which has been transformed with a construct encoding the GASA3 gene in sense direction, i.e. overexpressing GASA3.

5

Figure 11.

Formation of meristematic regions in the hypocotyl of *Arabidopsis thaliana* WS plants under influence of overexpression of RKS4.

10 RKS4 overexpression results in increases in flower and seed organ size that could be due to increase in cell elongation and/or cell division. In order to analyse the cell division patterns in plants with deregulated RKS4 expression the mitotic activity in transgenic plants was analyzed with the a
15 unstable GUS reporter under the control of a cyclin B1;1 promoter (the Plant Journal 1999 (4) 503-508 Spatio-temporal analysis of mitotic activity with a labile cyclin-GUS fusion protein). *Arabidopsis thaliana* WS seedlings with the pCDG construct did not show gus activity (cell division) in
20 hypocotyls (top) whereas the same pCDG line crossed with a constitutive RKS4 construct showed mitotic activity as indicated by GUS-positive cells (bottom); indicating that RKS4 overexpression activated mitotic activity in hypocotyls.

25 Figure 12

In *Arabidopsis thaliana* WS, the seed size is influenced by changing levels of RKS4 gene product. Constitutive overexpression of RKS4 results in increases in seed size (left) compared with control wildtype seeds (right). Antisense 30 constitutive expression of RKS4 cDNA (middle) results in a decrease in seed size compared with the control (right). Magnification is identical in all photos as shown by the bar size.

35

Figure 13

Organ size can be influenced by either modulating cell division or cell elongation or a combination of both. In order to identify the total number of cells and the cell size within an organ the apical site of petals of mature *Arabidopsis* flowers was investigated. Petal organ size is clearly influenced by modulation of RKS4 gene product levels (bottom row for the flowers from which the apical petal epidermal cells were identified). Epidermal cell size is not changed in transgenic plants compared with the control.

10

Figure 14

Arabidopsis thaliana WS plants in which the endogenous level of RKS10 gene product is increased (right picture) due to the presence of a transgenic RKS10 overexpressing construct. The left picture shows the apical epidermus of a full grown cotyl from an empty vector transgenic seedling of the same age as the transgenic overexpressing cotyl, grown under similar growth conditions..

20 Figure 15

Arabidopsis thaliana WS plants in which the endogenous level of RKS10 gene product is decreased (right picture) due to the presence of a RKS10 antisense construct. The left picture shows a wildtype plant of the same age as the transgenic antisense plant, grown under similar growth conditions. Plant size, organ size and number of organ primordia remains similar in both the transgenic antisense plants and the wildtype control.

30 Figure 16

In order to determine organ size variations in transgenic RKS10 transgenic plants compared with empty vector control transgenic plants (pGreen4K), flower organ size was determined of the four open flower stages of *Arabidopsis* inflorescences. The four successive flower stages are photographed under similar magnifications. No obvious changes in organ length could be observed in size of sepals, petals, stamen and carpel

between empty vector control flowers (pGreen4K), flowers with an antisense RKS10 construct (a) or plants overexpressing the RKS10 cDNA under the control of a 35S promoter (S

5 Figure 17

Tissue cultured auxin treated *transgenic Arabidopsis* T2 seedlings were grown on MS agar plates without hormones for a period of 3 weeks. Regeneration potential was scored and the formation and outgrowth of multiple shoot apical meristems

10 from single seedling origin was displayed as (+). The formation and outgrowth of only one shoot apical meristem, leading to the formation of a normal rosette of leaves from individual plants was displayed as (-). Positive regeneration controls consisted of seedlings overexpressing either KNAT1, 15 CUC2, IPT or cycD3. All of these showed an increase of regeneration capacity (+) compared with a negative control GUS overexpressing plant pGreen5K (-).

Representative examples of RKS and ELS cDNA overexpressing (s) or antisense (a) cosuppressing constructs in transgenic plants 20 are shown in the bottom panels.

Figure 18.

Tobacco leaf discs were stably transformed with the RKS0 overexpressing construct GT-RKS0-23S and from a single 25 transformation event, large numbers of regeneration plantlets were isolated and subcultured. All of the regenerated plants were potted and flowered. The original transformation event could be kept continuously in tissue culture indefinitely.

30 Figure 19

Seedlings from transgenic *Arabidopsis thaliana* containing either constructs overexpressing (s) or co-suppressing by antisense (a) the RKS gene products were screened for the 35 appearance of fasciation. Several examples in which fasciation could be routinely observed are shown together with a negative control plant (pGreen5K, overexpressing the GUS gene) in which fasciation could never be observed.

Figure 20 - 23

Primary root tips of transgenic *Arabidopsis* plants (top rows) photographed under similar magnification. The bottom rows show

5 the corresponding seedlings (also between each other under the same magnification). Figure 23 shows the specific *Arabidopsis* transgenes with a strong increase in root outgrowth.

Figure 24

10 Avarage root length of 10-30 transgenic *Arabidopsis* T2 seedlings from one T1 transgenic plant is shown.

Figure 25

15 T3 seedlings are shown from a strong co-suppressing RKS10 antisense construct line (T1-4; T2-6; T3 generation) and a strong overexpressing line (T1-4; T2-6; T3 generation). The overexpressing line is different and stronger from the one shown in Figure 4.1-4.5. Pictures are taken under similar magnifications.

20

Figure 26

T2 seed was germinated on horizontal MS agar plates and pictures were taken under similar magnification of representative examples of the lateral root development from 25 transgenic RKS and ELS transgenic roots.

Figure 27

Pictures taken from transgenic RKS8 or RKS10 overexpressing roots taken directly behind the tip zone. Pictures are taken 30 under same magnification.

Figure 28

Arabidopsis thaliana WS plants in which the endogenous level of RKS or ELS gene product is modified result in the formation 35 of new meristem formation and / or outgrowth, resulting in a complex, bushy inflorescence in transgenic *Arabidopsis* plants compared with control empty vector control plants (pGreen4K).

Overexpression of RKS10 and ELS1 (S) and cosuppression with antisense constructs of RKS8 and also RKS10, result in increased numbers of developing generative meristems. The generative shoots are photographed with similar 5 magnification.

Figure 29

Arabidopsis thaliana WS plants in which the endogenous level of RKS gene product is modified result in the formation of new 10 meristem formation and / or outgrowth, resulting in a complex, bushy inflorescence in transgenic *Arabidopsis* plants compared with control empty vector control plants (pGreen4K). The top panel shows adult plants under similar magnification. Compared with the control, RKS10 overexpression results in an extreme 15 bushy phenotypic plant. The results of co-suppressing the RKS8 gene product are less dramatic with respect to the bushiness. However, also in these transgenic plants the number of 20 generative meristems is strongly increased compared with the control. The bottom panel shows the generative shoot in detail under similar magnification.

Figure 30

Schematic drawing of the different flower organs in an empty vector control pGreen4K flower (left) compared with a complex 25 transgenic flower structure seen in transgenic *Arabidopsis* plants containing an antisense (a) RKS10 construct. The terminal flower meristem produces 2 sepals, 1 petal, 2 stamen, a carpel which is not a closed structure but open with groups of ovules on the inside and outside of this structure, and 30 stigmatic cells protruding from the top part. Two new flowers are protruding from this structure, containing all flower organs in normal numbers.

Figure 31

35 Schematic drawing of the different flower organs in a complex transgenic flower structure seen in transgenic *Arabidopsis* plants T1-11 containing an antisense (a) RKS10 construct. The

terminal flower meristem produces 1 sepal, 2 petals, 2 stamen, a carpel which is not a closed structure but open with groups of ovules on the inside and outside of this structure, and stigmatic cells protruding from the top part. An undetermined 5 flower meristem is protruding from the open carpel structure and forms a number of new flowers, including normal flowers (right) and another abnormal flower (left) which consists of a flower with half of the sepal, petal and stamen organs formed and a new terminal flower meristem protruding from this 10 structure, developing in structures as seen in Figure 7.5. The stamen contain only small numbers of (viable) pollen compared with wildtype stamen (see also chapter 5).

Figure 32

15 Schematic drawing of the different flower organs in an empty vector control pGreen4K flower (left) compared with a complex transgenic flower structure seen in a transgenic *Arabidopsis* plant T1-11 containing an antisense (a) RKS10 construct (overview shown in Figure 7.4). The terminal flower meristem 20 produces half the normal number of sepals, petals and stamen. The remaining part of the flower structure has converted into a new structure containing a new stem containing a single organ structure resembling a fusion between a petal and a sepal. On this structure several (viable) pollen grains can be 25 observed.

Figure 33

30 Schematic drawing of the different flower organs in a complex transgenic flower structure seen in a transgenic *Arabidopsis* plant T1-12 containing an antisense (a) RKS10 construct. The terminal flower meristem originating from an undetermined generative meristem is here producing an axillary secondary undetermined meristem (left picture), a single organ resembling a stamen (bottom left), a normal flower and a 35 terminal flower. This terminal flower structure contains 2 normal sepals, 2 normal petals, 2 normal stamen (with only a few viable pollen) and two organs resembling a fusion of

sepals /petals/stamen (see also figure 7.7). From this terminal flower structure two new flowers emerge (in a similar fashion as observed in Figure 7.3) containing normal numbers of flower organs (right photos). At the top of this figure a 5 control inflorescence is shown schematically with terminal flower meristems as normally originate from the generative *Arabidopsis thaliana* generative meristem.

Figure 34

10 Schematic drawing and detailed pictures of several of the structures as shown in figure 7.6. At the right the organs resembling a fusion between sepals/petals/stamen are shown with viable pollen sticking out from these structures. At the top left the single stamen-like organ directly protruding from 15 the main stem is shown.

Figure 35

Transgenic *Arabidopsis* plants overexpressing the RKS13 gene product show a modification of the normal flower inflorescence 20 architecture, somewhat resembling the structures observed in RKS10 antisense plants. A terminal flower containing a normal seed developing silique and a small number of sepals, petals and stamen, develops at least 4 additional terminal flower meristems that develop abnormally themselves, resulting in 25 open carpel structures and modifications of organ structures.

Figure 36

Transgenic plants in which the RKS and / or ELS genes are introduced behind a constitutive 35S promoter in an 30 overexpressing (S) or antisense (a) configuration are analyzed for sterility and characterized further for defects in proper pollen development. As a negative control the normal pollen development of a transgene containing the empty expression vector (pG4K) was included. First generation transgenic 35 flowers of RKS10 expressing constructs and second generation control vector and ELS2 are shown under similar magnification. In detail the stigmatic surface and surrounding stamen, are

104

shown under similar magnification, showing the presence or absence of pollen on the stamen or the stigmatic surface.

Detailed description

1. Modifying organ size

5

Plant size is determined by both cell elongation and cell division rate. Modifying either one or both processes results in a change in final organ size. Increasing the level of specific members of the family of RKS genes results in an 10 increase in organ size, growth rate and yield. Modulating plant growth, organ size and yield of plant organs is the most important process to be optimized in plant performance. Here we show that modulating the level of members of the family of the RKS signaling complex is sufficient to modulate these 15 processes. The invention provides herewith a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL 20 protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating cellular division during plant growth or organ formation, in particular wherein said gene comprises an RKS4 or RKJS 10 gene or functional equivalent thereof. Inactivation of endogenous RKS gene product results in a decrease in plant 25 growth, proving that the normal function of these endogenous RKS gene products is the regulation of growth and organ size. Elevation of the levels of the regulating of the RKS signaling complex in plant cells is provided in order to increase: the size of plant organs

30 the growth rate
the yield of harvested crop
the yield of total plant material
the total plant size

35 Decreasing the levels of endogenous RKS gene product is provided in order to decrease:
the size of plant organs

the growth rate
the total plant size

5

Results obtained (see also figures 6 to 13)

Overexpression and antisense constructs of full length RKS cDNA clones have been made under the control of 35S promoters. Transgenic plants have been produced in *Arabidopsis*

10 *thaliana* and in *Nicotiana tabacum*. Subsequent generations of stably transformed plants were investigated for phenotypes and analyzed in detail. The phenotype observed in transgenic plants with antisense constructs of RKS4 (GT-RKS4-a) could be

15 described as dwarf plants in which all plant organs showed a decrease in organs size and growth rate. Overexpression of RKS4 (GT-RKS4-s) resulted in plants with increased size of organs and an increase in growth rate. Since cell size alone was not responsible for the modifications in organ size of petals it can be concluded that RKS4 is involved in the

20 regulation of the cellular divisions during plant growth and organ formation. Overexpression of RKS 4 results in an increase of cellular divisions whereas a decrease in endogenous RKS 4 gene product levels within the plant results in a decrease of cellular division rates.

25

Literature

-Not being the wrong size. R.H. Gomer 2001; Nature reviews 2: 48-54

30 -Cell cycling and cell enlargement in developing leaves of *Arabidopsis*. P.M. Donnelly et al. 1999; Developmental biology 215: 407-419

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-Measuring dimensions: the regulation of size and shape. S.J. Day and P.A. Lawrence 2000; Development 127: 2977-2987

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2. Cell division

The mitotic cell cycle in eukaryotes determines the total number of cells within the organism and the number of cells within individual organs. The links between cell proliferation, cell differentiation and cell-cycle machinery are of primary importance for eukaryotes, and regulation of these processes allows modifications during every single stage of development. Here we show that modulating the level of members of the family of the RKS signaling complex is sufficient to modulate these processes. The invention provides herewith a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating cellular division during plant growth or organ formation, in particular wherein said gene comprises an RKS4 or RKJS 10 gene or functional equivalent. Herewith the invention provides a method for modulating the number of cells to be formed within an eukaryotic organism as a whole or for modulating the cell number within individual organs is, which of primary importance in modulating plant developmental processes, especially of arable plants. Here we show that members of the RKS signaling complex are able to regulate the number of cellular divisions, thereby regulating the total number of cells within the organism or different organs.

30 Possible Applications

Elevation of the levels of the regulating RKS signaling complex members in plant cells in order to increase:
the size of plant organs
the growth rate
35 the yield of harvested crop
the yield of total plant material
the total plant size

Decreasing the levels of endogenous RKS signaling complex members in order to decrease:

the size of plant organs

5 the growth rate

the total plant size

Results obtained

Overexpression and antisense constructs of full length RKS

10 cDNA clones have been made under the control of 35S promoters. Transgenic plants have been produced in *Arabidopsis thaliana* and in *Nicotiana tabacum*. Subsequent generations of stably transformed plants were investigated for phenotypes and analyzed in detail.

15 Overexpression of RKS 4 results in an increase of cellular divisions whereas a decrease in endogenous RKS 4 gene product levels within the plant results in a decrease of cellular division. Another example of RKS genes involved in cellular proliferation is provided by RKS10. Overexpression of RKS10 20 (S) results in a decrease in apical epidermal cells (Figure 14) compared with control plants containing an empty expression cassette (pGreen4K). Co-suppressing the endogenous RKS 10 gene in plants containing an antisense construct (a) showed clearly larger epidermal cells as the corresponding 25 cells in wildtype control plants (Figure 15). In contrast to the plant phenotypes shown in RKS4 transgenic plants, no differences in plant or organ size could be observed in the RKS10 transgenic plants or organs. This shows that although the organ size remains constant, the number of cells within 30 these organs is variable due to the differences in size of individual cells. These results indicate that normal RKS4 function within the plant can be described as an activator of cellular division.

Normal RKS10 function also involves an activation process on 35 cellular division rate. This effect is also detectable in the root in the region directly behind the tip zone, where in the RKS10 overexpressing transgenes cellular divisions were

detectable in a region where normally cell proliferation has ceased. The plane of divisions of root cells in these transgenes is also clearly different from the normal plane of root cell division, resulting in clumps of cells with all 5 types of division planes possible.

In contrast to RKS4, the final organ size in RKS10 transgenic plants is under the control of other organ size restriction processes, in such a way that the final organ volume remains constant (Figure 16). RKS4 and RKS10 are essentially involved 10 in the same cell cycle activation process, but either addition organ size controlling functions of these RKS genes or the hierarchical order in which they regulate the cell cycle is different.

15

Literature

- Not being the wrong size. R.H. Gomer 2001; Nature reviews 2: 20 48-54
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3. Regeneration

Modification the levels of different RKS and ELS genes within 5 plants allows the initiation and / or outgrowth of apical meristems, resulting in the formation of large numbers of plantlets from a single source. A number of gene products that is able to increase the regeneration potential of plants is known already. Examples of these are KNAT1, cycD3, CUC2 and 10 IPT. Here we show that modulation of the endogenous levels of RKS genes results in the formation of new shoots and plantlets in different plant species like *Nicotiana tabacum* and *Arabidopsis thaliana*. herewith the invention provides a method for modulating a developmental pathway of a plant or plant 15 cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein, allowing modulating apical meristem formation, in particular 20 wherein said gene comprises an ELS1, RKS0, RKS3, RKS4, RKS8 or RKS10 gene or functional equivalent thereof. A direct application of a method according to the invention is the stable or transient expression of RKS and ELS genes or gene 25 products in order to initiate vegetative reproduction.

Regeneration can be induced after overexpression of for 30 example RKS0 and ELS1; or by co-suppression of for example the endogenous RKS3, RKS4, RKS8 or RKS10 genes. Overexpression or co-suppression of these RKS and ELS gene products can be either transient, or stable by integration of the corresponding expression cassettes in the plant genome.

Results obtained

Overexpression and antisense constructs of full length RKS and ELS cDNA clones have been made under the control of 35S 35 promoters. Transgenic plants have been produced in *Arabidopsis thaliana* and in *Nicotiana tabacum*. Subsequent generations of

stably transformed plants were investigated for phenotypes and analyzed in detail.

T2 transgenic seedlings of *Arabidopsis* were germinated in liquid MS medium supplemented with 1 mg/L 2,4-D for 1 week,

5 followed by extensive washing and plating of the seedlings onto MS agar plates without hormones. Control transgenic seedstocks containing either a negative control vector (pGreen5K); or positive control overexpression constructs of gene products known to increase the regeneration potential

10 (IPT, KNAT1, CUC2 and cycD3) were characterized for regeneration potential together with seedstocks from plants either overexpressing (s) or co-suppressing (a) all RKS and ELS gene products (Figure 17). Overexpression of the ELS1 and RKS0 cDNA clones resulted in an increase of shoot apical

15 meristem formation and outgrowth, whereas antisense constructs (a) of these cDNA clones did not increase the regeneration potential (only increased regeneration results are shown).

Antisense constructs of RKS3, RKS4, RKS8 and RKS10 also resulted in an increased formation and outgrowth of apical

20 meristems (Figure 17).

T1 generation *Nicotiana tabacum* tissue cultures transformed with ELS and RKS gene products in either overexpression (s) cassettes or antisense co-suppression (a) cassettes allowed the regeneration of indefinite number of offspring plants from

25 a single transformed cell origin (Figure 18). An example is shown for the overexpression of the GT-RKS0-23S construct. The resulting plants obtained from one transformation event in general showed no phenotypes. Only a subset of plants displayed RKS0 overexpression phenotypes (like loss of apical dominance and early flowering).

Literature

-Mechanisms that control knox gene expression in the

35 *Arabidopsis* shoot. N. Ori et al. 2000, Development 127: 5523-5532

- Overexpression of KNAT1 in lettuce shifts leaf determinate growth to a shoot-like indeterminate growth associated with an accumulation of isopentenyltype cytokinins. G. Frugis et al. 2001. *Plant Physiology* 126: 1370-1380
- 5 -KNAT1 induces lobed leaves with ectopic meristems when overexpressed in *Arabidopsis*. Chuck et al. 1996. *the Plant Cell* 8: 1277-1289
- Cytokinin activation of *Arabidopsis* cell division through a D-type cyclin. C. Riou-Khamlich et al. 1999. *Science* 283: 10 1541-1544

4. Fasciation

Fasciation is normally a result from an increased size of the
5 apical meristem in apical plant organs.

Modulation of the number of cells within the proliferating
zone of the shoot apical meristem results in an excess number
of cellular divisions, giving rise to excess numbers of
primordia formed or to stems in which the number of cells is
10 increased.

The invention herewith provides a method for
modulating a developmental pathway of a plant or plant cell
comprising modifying a gene or modifying expression of said
gene, wherein said gene is encoding a protein belonging to a
signaling complex comprising RKS protein, ELS protein, NDR/NHL
15 protein, SBP/SPL protein and RKS/ELS ligand protein allowing
modulating fasciation, in particular wherein said gene
comprises an RKS0, RKS3, RKS8 or RKS10 gene or functional
equivalent thereof. Here we for example show that modulation
of the levels of RKS gene products in plants like *Arabidopsis*
20 *thaliana* can result in fasciated stems as shown in Figure 19.

A direct application as provided herein is the regulated
formation of fasciation in plant species in which such a trait
is desired like ornamental plants. Regulation of the
initiation and extent of fasciation, either by placing the
25 responsible RKS encoding DNA sequences under the control of
stage or tissue specific promoters, constitutive promoters or
inducible promoters results in plants with localized or
constitutive fasciation of stem tissue. Another application is
modulating the number of primordiae by regulation of the
30 process of fasciation. An example is provided by for example
sprouts, in which an increased number of primordia will result
in an increased numbers of sprouts to be harvested. Fasciation
can also result in a strong modification in the structural
architecture of the inflorescence, resulting in a terminal
35 group of flowers resembling the *Umbelliferae* type (an example
is shown in Figure 19 where the fasciated meristem of a RKS0-
7S *Arabidopsis* plant in which endogenous RKS0 gene product

levels have been deregulated clearly terminates in an *Umbelliferae* type inflorescence.

Results obtained

5 Overexpression and antisense constructs of full length RKS cDNA clones have been made under the control of 35S promoters. Transgenic plants have been produced in *Arabidopsis thaliana*. Subsequent generations of stably transformed plants were investigated for phenotypes and analyzed in detail.

10 T2 transgenic seedlings of *Arabidopsis* were germinated on MS agar plates without hormones. Control transgenic seedstocks containing a negative control vector (pGreen5K) were tested for their ability to induce fasciation (Overexpression constructs (s) of RKS0, RKS8 and RKS10 cDNA clones resulted in 15 fasciated plants, whereas antisense constructs (a) of these cDNA clones did not increase the regeneration potential (only positive results are shown). Antisense constructs of RKS3 gave also rise to fasciation (Figure 19).

20

Literature

-Functional domains in plant shoot meristems. U. Brand et al. 2001. *Bioassays* 23: 134-141.

25 -Dependence of stem cell fate in *Arabidopsis* on a feedback loop regulated by CLV3 activity.
U. Brand et al. 2000. *Science* 289: 617-619

5. Root development

Fasciation is normally a result from an increased size of the apical meristem in apical plant organs. Modulation of the

5 number of cells within the proliferating zone of the root apical meristem results in an excess number of cellular divisions, giving rise to excess numbers of primordia formed or to roots in which the number of cells is increased.

Adaptation to soil conditions is possible by regulation of 10 root development of plants. Here we describe several processes in root development that can be manipulated by modification of the levels of the RKS signaling complex within the root. The invention provides a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene

15 or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein allowing modulating root development,

in particular wherein said gene comprises an ELS1, ELS2, RKS1, 20 RKS3, RKS4, RKS6 RKS8 or RKS10 gene or functional equivalent thereof. Root length, a result by either root cells

proliferation or elongation, can for example be increased by overexpression of for example RKS3, RKS4, RKS6 and ELS2, or inactivation of the endogenous RKS10 gene product. Root length

25 can also be decreased by decreasing of endogenous RKS1 levels or by strong overexpression of RKS10. The initiation of lateral roots is also regulated by RKS gene products.

Overexpression of for example RKS10 can result in a strong increase in the initiation and outgrowth of lateral roots. Co- 30 suppression of RKS1 also resulted in the initiation and outgrowth of large numbers of lateral roots. Root hair

formation and elongation is important in determining the total contact surface between plant and soil. A strong increase of root hair length (elongation) can be obtained by

35 overexpression of ELS1 and RKS3 gene products. As the roots of terrestrial plants are involved in the acquisition of water and nutrients, anchorage of the plant, synthesis of plant

hormones, interaction with the rhizosphere and storage functions, increasing or decreasing root length, for example for flexible adaptations to different water levels, can be manipulated by overexpressing or cosuppressing RKS and / or 5 ELS gene products. Modulation of the total contact surface between plant cells and the outside environment can be manipulated by regulation lateral root formation (increased by RKS10 overexpression and co-suppression of RKS1). Finally the contact surface between plant cells and the soil can be 10 influenced by modulation of the number of root hairs formed or the elongation of the root hairs, as mediated by ELS1 and RKS3.

Results obtained

15 Overexpression and antisense constructs of full length RKS cDNA clones have been made under the control of 35S promoters. Transgenic plants have been produced in *Arabidopsis thaliana*. Subsequent generations of stably transformed plants were investigated for phenotypes and analyzed in detail.

20 T2 transgenic seedlings of *Arabidopsis* were germinated on MS agar plates without hormones. Control transgenic seedstocks containing a negative control vector pGreen4K (empty expression vector) and / or pGreen5K (a GUS overproducing vector) were included as references for normal root

25 development. Seedlings from transgenic *Arabidopsis thaliana* containing either constructs overexpressing (s) or co-suppressing by antisense (a) the RKS gene products were screened for the appearance of fasciation. Several examples in which fasciation could be routinely observed are shown

30 together with a negative control plant (pGreen4K, containing an expressing cassette without an insert cDNA). Seedlings are germinated and grown on vertically placed MS agar plates.

-Cellular organisation of the *Arabidopsis thaliana* root. L.

Dolan et al. 1993. Development 119: 71-84

-Root development in *Arabidopsis*: four mutants with dramatically altered root morphogenesis. P.N. Benfey et al.

5 1993. Development 119: 57-70

-The development of plant roots: new approaches to underground problems. J.W. Schiefelbeim and P.N. Benfey 1991. the Plant Cell 3: 1147-1154

6. Apical meristems

All parts of the plant above the ground are generally the result on one apical shoot meristem that has been initiated 5 early at embryogenesis and that gives rise to all apical organs. This development of a single meristem into complex tissue and repeated patterns is the result of tissue and stage-dependent differentiation processes within the meristems and its resulting offspring cells. The control of meristem 10 formation, meristem identity and meristem differentiation is therefore an important tool in regulating plant architecture and development. Here we present evidence the function of RKS and ELS gene products in regulation of the meristem identity and the formation and outgrowth of new apical meristems. The 15 invention provides a method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and 20 RKS/ELS ligand protein allowing modulating meristem identity, in particular wherein said gene comprises an ELS1, RKS8, RKS10 or RKS13 gene or functional equivalent thereof. Introduction of for example the RKS10 gene product or an other member of the RKS signaling complex under the control of a tissue and / 25 or stage specific promoter as provided herein allows localized and time regulated increases in the levels of gene product. For example the meristematic identity in a determined meristem might thereby be switched back into an undetermined meristem, thereby changing for example a terminal flower into an 30 undetermined generative meristem.

Another application might be found in changing the meristematic identity at an early time point, during early vegetative growth, thereby switching the vegetative meristem into a generative meristem, allowing early flowering.

35 Modulation of meristem identity in terminal primordia, like for example as shown in Figure 30, where flower organ primordia are converted into terminal flower primordia, allows

the formation of completely new types of flowers and fused fruit structures. Constitutive overexpression of RKS gene products results in plants with many apical meristems, as can clearly been seen in Figure 29, where RKS10 overexpression 5 results in an extremely bushy phenotype.

Results obtained

Changing the normal levels of endogenous RKS10 within the 10 plant, either by overexpressing or co-suppressing the RKS10 cDNA, results in an increase in generative meristem development (Figure 28).

Compared with the control empty vector transgenic pGreen4K plants, large number of meristems are initiated at places were 15 normally no meristems initiate and / or develop. A clear example is shown by co-suppressing the RKS8 gene (Figure 29), where many new inflorescence meristems are initiated from the central generative meristem compared with control pGreen4K plants of the same age. This phenotype is even more extreme in 20 RKS10 overexpressing plants where the resulting plants are extremely bushy with very large numbers of generative meristems formed. Inactivation of the endogenous RKS10 gene in *Arabidopsis* results in modification of meristematic identity as can be shown in Figure 30. A determined flower meristem 25 develops into two new normal terminal flower meristems and a number of terminal flower organ primordia. Another example is shown in Figure 31 where meristem determination is switched from a terminal flower meristem, that normally result only in the normal numbers of terminal organ primordia, towards a 30 number of organ primordia, a new undetermined generative meristem that develop into normal flowers or in a new terminal flower meristem with developmental abnormalities. Only half of the terminal flower primordia develop normally while an extra structure arises resembling a new flower stem with a 35 petal/stamen like organ. The few pollen detectable on this structure (Figure 32) were able to pollinate a MS1 (male sterile) *Arabidopsis* flower. Figure 33 shows the meristematic

developmental switch from a terminal flower meristem into a new undetermined generative meristem, that gives rise to a new formation of another undetermined meristem, and several normal and abnormal terminal flowers. The abnormal flowers again show 5 the fusion of different structures, in this case from sepals, petals and stamen together (Figure 34). Surprisingly, directly on the generative stem another structure, resembling a single stamen was detectable. All these data indicate that a decrease in RKS1 expression levels results in switches in the 10 meristematic identity. Meristems can switch forward and backward between developmental stages, indicating that RKS10 is normally involved in regulating the meristematic identity and the developmental order of meristematic development. RKS13 seems to be involved in similar processes, as can be concluded 15 from the switches in flower meristematic outgrowths observed in figure 35. Modification of the expression levels of RKS1 also results in modified meristem identity. Suppression of endogenous RKS1 levels results in a developmental switching of generative meristems towards vegetative meristems, together 20 with other phenotypes (results not shown).

Literature

- To be, or not to be a flower-control of floral meristem 25 identity. H. Ma 1998. Trends in Genetics 14: 26-32
- A genetic framework for floral patterning. F. Parcy et al. 1998 Nature 395: 561-566
- Evolution of flowers and inflorescences. E.S. Coen and J.M. Nugent 1994. Development supplement 107-116
- 30 -Control of shoot cell fate: beyond homeoboxes. M. Tsiantis 2001. the Plant Cell 13: 733-738
- Floral induction and determinations: where is flowering controlled? F.D. Hempel et al. 2000. Trends in plant science 5: 17-21
- 35 -The *Arabidopsis* compact inflorescence genes: phase-specific growth regulation and the determination of inflorescence

architecture. L. Goosey and R. Sharrock 2001. the Plant
Journal 26: 549-559.

7. Male sterility

Male sterility is a highly desired trait in many plant species. For example, manipulation of pollen development is crucial for F1 hybrid seed production, to reduce labour costs and for the production of low-environmental impact genetically engineered crops. In order to produce hybrid seed from inbred plant lines, the male organs are removed from each flower, and pollen from another parent is applied manually to produce the hybrid seed. This labour-intensive method is used with a number of vegetables (e.g. hybrid tomatoes) and with many ornamental plants. Transgenic approaches, in which one or more introduced gene products interfere with normal pollen initiation and development is therefore highly desired.

Especially when the number of revertants (growing normal pollen) is extremely low.

Male sterility in plants is a desired trait that has been shown already in many plant species as a result of the inactivation of expression of a number of genes essential for proper stamen development, mitotic divisions in the pollen stem cells, or male gametogenesis. A method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein, allowing modulating pollen development, in particular wherein said gene comprises an ELS2 or RKS10 gene or functional equivalent thereof.

Here we present data that show that overexpression of gene products, like transmembrane receptor kinases (RKS) and extracellular proteins (ELS) can also result in the formation of male sterility. The ability to induce male sterility by overexpressing specific genes as provided herein allows the opportunity to produce transgenic overexpressing plants in which the pollen development is inhibited. Stable single copy homozygous integration of such overexpressing traits into the

plant genome will render such plants completely sterile, making them excellent material for the production of F1 hybrid seed. Furthermore, the combined integration of a male sterility inducing overexpressing gene coupled directly with

5 another desired transgene result in transgenic plants which are unable to produce transgenic seed, making these transgenic plants excellent material for outside growth without problems affecting transgenic pollen spreading throughout the environment, thereby eliminating possible crosses

10 with wild plant species or other non-transgenic crops. The combination of a desired transgene flanked on both sites by different male-sterility inducing overexpressing genes would decrease the frequency of pollen formation to an extremely low level. An example is an overexpressing construct of RKS10

15 at the 5'end of integrated DNA fragment, the desired transgene expression cassette in the middle and at the 3'end of the integrated DNA the ELS2 overexpressing construct. This complete DNA fragment is integrated into the genome by conventional techniques, like particle bombardment,

20 *Agrobacterium* transformation etc. Another possible application concerns the modification of pollen in ornamental plant species like lily, where the release of pollen from cut flowers can be avoided by making transgenic plants in which pollen development is initiated by release from the stamen is 25 prevented (a desired trait that can be obtained by overexpressing for example ELS2, resulting in partial pollen development). Hereby the ornamental value of the stamen with pollen is not lost, but release of pollen is inhibited.

30 **Results obtained**

Overexpression and antisense constructs of full length RKS cDNA clones have been made under the control of 35S promoters. Transgenic plants have been produced in *Arabidopsis thaliana*. Subsequent generations of stably transformed plants 35 were investigated for phenotypes and analyzed in detail. T2 transgenic seedlings of *Arabidopsis* were germinated on MS agar plates without hormones. Control transgenic plants

containing a negative control vector pGreen4K (empty expression vector) were included as references for normal stamen and pollen development. RKS10 and ELS2 resulted in sterile plants when overexpressed in *Arabidopsis*. Antisense 5 RKS10 plants resulted in a strong reduction in the number of pollen formed (Figure 36). In order to determine whether pollen development itself was the reason for sterility (and not a combination of pollen developmental mutants coupled to either embryo lethals or female gametogenesis defects), 10 reciprocal crosses were performed between sterile transgenic plants and wildtype *Arabidopsis thaliana* WS plants. These results confirmed that the sterile plants with overexpressing RKS10 and ELS2 constructs were male sterile but completely female fertile. No defects could be observed in embryo 15 development from crosses between female transgenic overexpressors and male wildtype pollen (results not shown). Since both antisense and overexpressing constructs of the RKS10 gene showed defects in proper pollen development we conclude that normal levels of endogenous RKS10 gene product 20 are essential for proper pollen formation, outgrowth and differentiation. In the ELS2 overexpressing plants the initiation of pollen grains was not inhibited. However the proper development of pollen grains in full grown viable pollen was clearly inhibited .

25

Literature

-The *Arabidopsis* male sterility1 (MS1) gene is a transcriptional regulator of male gametogenesis, with homology 30 to the PHD-finger family of transcription factors. Wilson et al. 2001. the Plant Journal 28: 27-39
-Transposon tagging of a male sterility gene in *Arabidopsis*. Aarts et al. 1993. Nature 363: 715-717

35

8. Resistance mechanisms

Two-hybrid interaction experiments have already shown *in vitro* interaction between RKS and NDR0-NHL and members of the

5 SBP/SPL family. Here we show that *in vivo* the individual components of this signalling cascade are regulating identical processes, as based on functional genomics on transgenics plants, overexpressing or co-suppressing single components or combinations of components in this transmembrane signalling

10 complex.

Here we show a large number of new members of the NDR/NHL gene family and we postulate a function as syntaxins in the pathogen resistance:

15 **At2g27080;**

MAERVYPADS PPQSGQFSGN FSSGEFPKKP APPPSTYVIQ VPKDQIYRIP PPENAHRFEQ
 LSRKKTNRSN CRCCFCFSFLA AVFILIVLAG ISFAVLYLIY RPEAPKYSIE GFSVSGINLN
 STSPISPSFN VTVRSRNGNG KIGVYYEKES SVDVYYNDVD ISNGVMPVFY QPAKNVTVVK
 LVLSGSKIQL TSGMRKEMRN EVSKKTVPK LKIKAPVKIK FGSVKTWTMI VNVDCDVTVD
 20 KLTAPSRIVS RKCSHDVDLW **

At5g21130

MTVEKPQEMT GDTNSDGFLT NKDVHRIKHP SLDTNDSSSS RYSVDSQKSR IGPPPGTYVI
 KLPKDQIYRV PPPENAHRYE YLSRRKTNKS
 25 CCRRCLCYSL SALLIIIVLA AIAFGFFYLV
 YQPHKPQFSV SGVSVTGINL TSSSPFSPVI RIKLRSQNVK GKLGLIYEKG NEADVFFNGT
 KLGNGEFTAF KQPAGNVTVI VTVLKGSSVK LKSSSRKELT ESQKKGKVPF GLRIKAPVKF
 KVGSVTTWMT TITVDCKITV DKLTASATVK TENCETGLSL L*

30 **At1g65690**

MSQHQKIYPV QDPEAATARP TAPLVPRGSS RSEHGDPSKV PLNQRPQRFFV PLAPPKKRRS
 CCCRCFCYTF CFLLLLVVAV GASIGILYLV FKPKLPDYSI DRLQLTRFAL NQDSSLTTAF
 NVTITAKNPN EKIGIYYEDG SKITVWYMEH QLSNGSLPKF YQGHENTIVI YVEMTGQTQN
 35 ASGLRTTLEE QQQRTGNIPL RIRVNQPVRV KFGKLKLFEV RFLVRGCVFV DSLATNNVIK
 IQSSSCKFRL RL*

At5g36970

MSDHQKIHPV SDPEAPPHPT APLVPRGSSR SEHGDPTKTQ QAAPLDPPRE KKGSRS
 CWCRCVCYTLVLF LLIVIVGAIV GILYLVFRPK FPDYNIDRLQ LTRFQLNQDL
 40 SLSTAFNVII
 TAKNPNEKIG IYYEDGSKIS VLYMQTRISN GSLPKFYQGH ENTTIILVEM TGFTQNATSL
 MTTLQEQQRL TGSIPLRIRV TQPVRIKLGK LKLMKVRFLV RCGVSVDLSA ANSVIRVRSS
 NCKYRFLR*

45 **At1g54540**

MGDQQKIHPV LQMEANKTKT TTPAPGKTVL LPVQRPIPPP VIPSKNRNMC CKIFCWVLSL
 LVIALIALAI AVAVVYFVFH PKLPSYEVNS LRVTNLGINL DLSLSAEFKV EITARNPNEK
 IGIYYEKGGH IGVWYDCKKL CEGPIPRFYQ GHRNVTKLNV ALTGRAQYGN TVLAALQQQQ
 QTGRVPLDLK VNAPVAIKLG NLKMKKIRIL GSCKLVDSDL STNNNNNIKA SDCSFKAKL*

50

At5g06320

MADLNGAYYG PSIPPPKKVS HSHGRRGGGC GCLGDCLGCC GCCILSVIFN ILITIAVLLG
 IAALIILWIF RPNAIKFHVT DAKLTEFTLD PTNNLRYNLD LNFTIRNPNR RIGVYVDEIE
 VRGYYGDQRF GMSNNISKFY QGHKNTTVVG TKLVGQQLVL LDGGERKDLN EDVNSQIYRI
 DAKLRLKIRF KFGLIKSWRF KPKIKCDLK VPLTSNSTSGF VFQPTKCDVD F**

5

At5g11890

MTDRVFPASK PPTATNGAPP VGSIPPPPAP ATVTSNGTTN GMANQKPQVY IPANRPVYRP
 QPYSRHHHQ SRPSCRRICC CCCFWSILII LILALMTAIA ATAMYVIYHP RPPSFVPSI
 RISRVNLTT SDSSVSHLSS FFNFTLISEN PNQHLSFSYD PFTVTVNSAK SGTMLGNGTV
 10 PAFFSDNGNK TSFHGVIATS TAARELDPDE AKHLRSDLTR ARVGYEIEMR TKVKMIMGKL
 KSEGVEIKVT CEGFEGTIPK GKTPIVATSK KTKCKSDLSV KVWKWSF*

At1g17620

MTDDRVYPAS KPPAIVGGGA PTTNPTFPAN KAQLYNANRP AYRPPAGRRR TSHTRG
 15 CCCRCCCWTIFVII LLLLIVAAAS AVVYLIYRPQ RPSFTVSELK ISTLNFTSAV
 RLTTAISLSV
 IARNPNKNVG FIYDVTDTIL YKASTGGDDD VVIGKGTIAA FSHGKKNTTT LRSTIGSPPD
 ELDEISAGKL KGDLKAKKAV AIKIVLNSKV KVKGALKTP KSGIRVTCEG IKVVAPTGKK
 ATTATTSAAK CKVDPRFKIW KITF**

20

At3g11650

MGSKQPYLNG AYYGPSIPPP PKAHRSYNSP GFGCCCFSCL GSCLRCCGCC ILSLICNILI
 AVAVILGVAA LILWLIFRPN AVKFYVADAN LNRFSFDPNN NLHYSLDLNF TIRNPNQRVG
 25 VYVYDEFSVSG YYGDQRFGSA NVSSFYQGHK NTTVILTKIE GQNLVVLGDG ARTDLKDEK
 SGIYRINA KL RLSVRFKFWF IKSWKLKP KI KCDDLKIPLG SSNSTGGFKF QPVQCDFDLS**

At2g22180

MEGPRRPPSA TAPDSDDDKP DDPPSVWHRP TSSLPALPSL DPPSHGSHHW RNHSLNLSPL
 30 PTTSSPPLPP PDSIPELETY VVQVPRDQVY WTPPPEHAKY VEKRSKNPEK NKKKGCSKRL
 LWFFIILVIF GFLLGAIILY LHFAFNPTLP VFAVERLTVN PSNFEVTLRA ENPTSNMGVR
 YMMEKNGVVS LTYKNKSLGS GKFPGLSQAA SGSDKVNVL NGSTKNAVQ PRGSKQPVVL
 MLNMELKA EAGPVKRNE VVTC DVKVVK GLDAKKVEI VSENCESEFK N*

35

At5g22870

MCHKPKLELM PMETSPAQPL RRPSLICYIF LVILTLIFMA AVGFLITWLE TKPKKLRYTV
 ENASVQNFNL TNDNHMSATF QFTIQSHNPN HRISVYYSSV EIFVKFKDQT LAFDTVEPFH
 QPRMNVKQID ETLIAENVA SKSNGKDLRS QNSLGKIGFE VFVKARVRFK VGIWKSSHRT
 AKIKCSHVT SLSQPNKSQN SSCDADI*

40

At2g35980

MAAEQPLNGA FYGPSVPPPA PKGYYRRGHG RGCGCCLLSS FVVKIISLIV ILGVAALIFW
 LIVR PRAIKF HVTDASLTRF DHTSPDNILR YNLALTVPVR NPNKRIGLYY DRIEAHAYYE
 45 GKRFSITLT PFYQGHKNTT VLTPTFQGQN LVIFNAGQSR TLNAERISGV YNIEIKFRLR
 VRFKLGDLKF RRIKPKVDCD DLRLPLSTSN GTTTSTVFP IKCDFD**

At2g46300

MADYQMN PVL QKPPGYRDPN MSSPPPPPPP IQQQPMRKAV PMPTS YRPKK KRRSCCRFCC
 CCICITLVLF IFLLL VGTAV FYLWFDPKLP TFSLASFRLD GFKLADDPDG ASLSATAVAR
 50 VEMKNPNSKL VFYYGNTAVD LSVGGSGNDET GMGETTMNGF RQGPKNSTSV KVETTVKNQL
 VERGLAKRLA AKFQS KDLVI NVVAKTKVGL GVGIGKIGML AVNLRCGGVS LNKLD TDSPK
 CILNTLK WYK IISN*

At4g05220

55 MTPDRTTIPI RTSPV PRAQP MKRHSASYY AHRVRESLST RISKFICAMF
 LLVLFFVGVVI AFILWLSLRP HRPRFHIQDF

VVQGLDQPTG VENARIAFNV TILNPQNQHMG VYFDSMEGSI YYKDQRVGLI
 PLLNPFFQQP TNTTIVTGTG TGASLTVNSN RWTEFSNDRA QGTVGFRLDI
 VSTIRFKLHR WISKHHRMHA NCNIVVGRDG LILPKFNHKR CPVYFT*

5 **At2g35460**

MANGLNGASY GPPIKPPVKT YYSHGRRGSD VGCGICGCFS SCLLCGGCL VNIICNILIG
 VLVCLGVVAL ILWFILRPNV VKFQVTEADL TRFEFDPRSH NLHYNISLNF SIRNPNQRLG
 IHYDQLEVRG YYGDQRFSAA NMNTSFYQGHK NTTVVGTELN GQKLVLLGAG GRRDFREDRR
 SGVYRIDVKL RFKLRFKFGF LNSWAVRPKI KCHLKVPPLST SSSDERFQFH PTKCHVDL*

10

At2g27260

MQDPSRPATG YPYPPYPNP QQQQPPTNGY PNPAAGTAYP YQNHNPYYAP QPNPRAVIIR
 RLFIVFTTFL LLLGLLIFIF FLIVRPQLPD VNLSNSLSVSN FNVSNNQVSG KWDLQLQFRN
 PNSKMSLHYE TALCAMYYNR VSLSETRLQP FDQGKKDQTV VNATLSVSGT YVDGRLVDSI
 15 GKERSVKGNV EFDLRMISYV TFRYGAFFFF RYVTVYCDDV AVGVPVSSGE GKMVGSSKRC
 KTY**

At4g01410

MGEGEAKAEH AAKADHKNAP SASSTPESYS KEGGGGGGDA RRAICGAIFT ILVILGIIAL
 20 ILWLWYRPHK PRLTVVGAAI YDLNFTTAPPL ISTSVQFSVL ARNPNRRVSI HYDKLSMYVT
 YKDQIITPPL PLPPLRLGHK STVVIAPVMG GNGIPVSPEV ANGLKNDEAY GVVLMRVVF
 GRLRWKAGAI KTGRYGFYAR CDVWLRFNPS SNGQVPLLAP STCKVDV*

At5g22200

25 MTGGRYCDQHN GYEERRMRMM MRRIAWACLG LIVAVAFVVF LVWAILHPHG PRFVLQDVTI
 NDFNVSQPNF LSSNLQVTVS SRNPNDKIGI FYDRLDIYVT YRNQEVTLAR LLPSTYQGHL
 EVTVWSPFLI GSAVPVAPYL SSALNEDLFA GLVLLNIKID GWVRWKVGSW VSGSYRLHVN
 CPAFIVTVGK LTGTGPAIKY QLVQRCAVDV *

30 **At1g61760**

MHNKVDSDLV RSNPSTRPIS RHHSASNIHV RVKESLTTRV SKLICAIFLS LLLCLGIITF
 ILWISLQPHR PRVHIRGFSI SGLSRPDGFE TSHISFKITA HNPQNQVGIY YDSMEGSVYY
 KEKRIGSTKL TNPFYQDPKN TSSIDGALSR PAMAVNKDRW MEMERDRNQG KIMFRLKVR
 MIRFKVYTWH SKSHKMYASC YIEIGWDGML LSATKDKRCP VYFT*

35

At3g52470

MSKDCGNHGG GKEVVVRKLC AAIIAFIVIV LITIFLVWVI LRPTKPRFVL QDATVYAFNL
 SQPNLLTSNF QVTIASRNPN SKIGIYYDRL HVYATYMNQQ ITLRTAIPPT YQGHKEVN
 40 SPFVYGTAVP IAPYNSVALG EEKDRGFVGL MIRADGTVRW KVRTLITGKY HIHVRCQAFI
 NLGNKAAGVL VGDNAVKYTL ANKCSVNV**

At5g53730

45 MSQISITSPK HCAKKGGINI NNRHKKLFFT FSTFFSGLLL IIFLWVLILH PERPEFSLTE
 ADIYSLNLTT SSTHLLNSSV QLTLFSKNPN KKVGIIYDKL LVYAAYRGQQ ITSEASLPPF
 YQSHEEINLL TAFLQGTELP VAQSFQYQIS RERSTGKIII GMKMDGKLRW KIGTVVSGAY
 RFNVNCLAIV AFGMNMTTPP LASLQGTRCS TTI*

At4g01110

50 MAGETLLKPV LQKPPGYREL HSQPQTPLGS SSSSSSMLRR PPKHAIPAAF YPTKKRQWSR
 CRVFCCCVCI TVAIVILLI LTVSVFFLYY SPRLPVVRLS SFRVSNFNFS GGKAGDGLSQ
 LTAEATARLD FRNPNGKLRY YYGNVDVAVS VGEDDFETSL GSTKVKGFVE KPGNRTVVIV
 PIKVKKQQVD DPTVKRLRAD MKSKKLVVKV MAKTKVGLGV GRRKIVTVGV TISCGGVRLQ
 TLDISKMSKCT IKMLKWyVPI QVKCI*

55 **At2g35960**

MTTKDCGNHG GGGGGGTASR ICGVIIGFII IVLITIFLVW IILQPTKPRF ILQDATVYAF

NLSQPNLLTS NFQITIASRN RNSRIGIYYD RLHVYATYRN QQITLRTAIP PTYQGHKEDN
 VWSPEVYGN S VPIAPFNAVA LGDEQNRGFV TLJIRADGRV RWKVGTLITG KYHLHVRCQA
 FINLADKAAG VHVGNAVKY MLINKCSVNV *

5 **At3g52460**

MPSPPEETQ PKPDTGPGQN SERDINQPPP PPPQSQPPP QTQQQTYPPV MGYPGYHQPP
 PPYPNYPNAP YQQQPYAQAP PASYYGSSYP AQQNPVYQRP ASSGFVRGIF TGLLIVLVLL
 CISTTITWLV LRPQIPLFSV NNFSVSNFNV TGPVFSAQWT ANLTIENQNT KLKGYFDRIQ
 GLVYHQNAV G EDEFLATAFF QPVFVETKKS VVIGETLTAG DKEQPKVPSW VVDEMKKERE
 10 TGTVTFSLRM AWWVTFKTDG WAARESGLKV FCGKLKVGFE GISGNGAVLL PKPLPCVVYV*

At4g09590

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 FNLSQPNLLT SKFQITIASR NRNSNIGIYY DHLHAYASYR NQQITLASDL PPTYQRHKED
 15 SVWSPLLYGN QVPIAPFNAV ALGDEQNSGV FTLTICVDGQ VRWKVGLTLI GNYHLHVRCQ
 AFINQADKAA GVHVGENTVK YTLINKCSVN F*

At2g35970

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 20 FNLSQPNLLT SKFQITIASR NRNSNIGIYY DHLHAYASYR NQQITLASDL PPTYQRHKEN
 SVWSPLLYGN QVPIAPFNAV ALGDEQNSGV FTLTICVDGR VRWKVGLTLI GNYHLHVRCQ
 AFINQADKAA GVHVGENTVK YTLINKCSVN F*

At3g26350

25 MSHHHHHETN PHFARIIPSQN PHLKSGGAST SQTSSNQPHI PPIPHPKKSH HKTTQPHPVA
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 PPPQAPQRNG GGYGSTLPPi PKPSPWRTAP TPSPHRRGP RLPPPSRETN AMTWSAAFCC
 AIFWVILILG GLIILIVYLV YRPRSPYVDI SAANLNAAYL DMGFLNGDL TILANVTNPS
 30 KKSSVEFSYV TFELYYYNTL IATQYIEPK VPKKTSMFAN VHLVSSQVQL QATQSRELQR
 QIETGPVLLN LRGMFHARSH IGPLFRYSYK LHTHCSVSLN GPPLGAMRAR RCNTKR*

At3g11660

MKDCENHGS RRKLIRRIFW SIIIFVLFIIF LTILLIWAIL QPSKPRFILQ DATVYAFNVS
 GNPPNLLTSN FQITLSSRNP NNNKIGIYYDR LDVYATYRSQ QITFPTSIPP TYQGHKDVDI
 35 WSPFVYGTsv PIAPPNGVSL DTDKDNGVVL LIIRADGRVR WKVGTFITGK YHLHVVKCPAY
 INFGNKANGV IVGDNNAVKYT FTTSCSVSV**

At3g44220

40 MTEKECEHHH DEDEKMRKRI GALVLGFLAA VLFFFVFLWA ILHPHGPRFV
 LQDATIYAFN VSQPNYLTNSN LQVTLSSRNP NDKIGIFYDR LDIYASYRNQ
 QVTLATLLPA TYQGHLDVTI WSPFLYGTv PVAPYFSPAL SQDLTAGMVL
 LNIKIDGWVR WKVGTWVSGR YRLHVNCPAY ITLAGHFSGD GPAVKYQLVQ RCAVDV*

At1g08160

45 MVPPNPAHQP ARRTQPQLQP QSOPRAQPLP GRRMNPVLCI IVALVLLGLL VGLAILITYL
 TLRPKRLIYT VEAASVQEFA IGNNDDHINA KFSYVIKSYN PEKHVSRYH SMRISTAHHN
 QSVAHKNISP FKQRPKNETR IETQLVSHNV ALSKFNARDL RAEKSKGTIE MEVYITARVS
 YKTWIFRSRR RTLKAVCTPV MINVTSSSLD GFQRVLCKTR L**

50 **At2g01080**

MPPPPSSSSRA GLNGDPIAAQ NQQPYYRSYS SSSSASLKGCCCLFLLFAF LALLVLAVVL
 IVILAVKPKK PQFDLQQVAV VYMGISNPSA VLDPTTASLS LTIRMLFTAV NPNKVGIRYG
 ESSFTVMYKG MPLGRATVPG FYQDAHSTKN VEATISVDRV NLMQAHAAIDL VRDASLNDRV
 ELTVRGDVGA KIRVMNFDSP GVQVLLPSFL PAFCSLSDL *

55

At5g06330

MTSKDCGSHD SHSSCNRKIV IWTISIILLL ILVVVILLVWA ILQPSKPRFV LQDATVFNFN
 VSGNPPNLLT SNFQFTLSSR NPNDKIGIYY DRLDVYASYR SQQITLPSPM LTTYQGHKEV
 NVWSPFVGGY SVPVAPYNAF YLDQDHSSGA IMLMLHLDGR VRWKVGFSFIT GKYHLHVRCH
 ALINFGSSAA GVIVGKMLT ETCSVSV*

5

At5g56050

MSKFSPPPQS QPQPPETPPW ETPSSKWYSP IYTPWRTTPR STQSTPTTTP IALTEVIVSK
 SPLSNQKSPA TPKLDMSMEAH PLHETMVLLQ LRTSRTNPWI WCGAALCFIF SILLIVFGIA
 TLILYLAVKP RTPVFDISNA KLNTILFESP VYFNGDMLLQ LNFTNPNKKL NVRFENLMVE
 10 LWFADTKIAT QGVLPSQRN GKTRLEPIRL ISNLVFLPVN HILELRRQVT SNRIAYEIRS
 NFRVKAIFGM IHYSYMLHGI CQLQLSSPPA GGLVYRNCTT KRW*

At3g20600**NDR1**

15 MNNQNEDTEG GRNCCTCCLS FIFTAGLTSF FLWLSLRADK PKCSIQNFFI PALGKDPNSR
 DNTTLMFMR CDNPNKDKGI YYDDVHLNFS TINTTKINSS ALVLVGNYTV PKFYQGHKKK
 AKKWGQVKPL NNQTVLRAVL PNGSAVFRLD LKTQVRFKIV FWKTKRYGVE VGADVEVNGD
 GVKAQKKGK MKKSDSSFPL RSSFPISVLM NLLVFFAIR*

20

At3g54200

MSDFSIKPDD KKEEEKPATA MLPPPKNAS SMETQSANTG TAKLRRKRM CKICICFTIL
 LILLIAIVIV ILAFTLFKPK RPTTIDSVD VDRLQASVNP LLLKVLLNLT LNVDSLKNP
 NRIGFSYDSS SALLNYRGQV IGEAPLPANR IAARKTVPLN ITLTLMAADRL LSETQLLSDV
 MAGVIPLNTF VKVTGKVTVL KIFKIKVQSS SSCDLSISVS DRNVTQHCK YSTKL*

25

At3g20590

non-race specific disease resistance protein, putative
 MTKIDPEEEL GRKCCTCFFK FIFTTRLGAL ILWLSLRACK PKCSIQNFYI PALSKNLSSR
 DNTTLMFMR CDNPNKDKGI YYDDVHLTFS TINTTTTNSD DLVLVANYTV PKFYQGHKKK
 30 AKKWGQVWPL NNQTVLRAVL PNGSAVFRLD LKTHVRFKIV FWKTKWYRRI KVGADVEVNG
 DGVKAQKKGK KTKKSDSSLP LRSSFPIFVL MNLLVFFAIR *

At4g39740

35 MSHVTATSLA RFTKPVPKPA SSPIVNTKLT TSGGRTAAFM DLSSFRLTVW
 DPDTANDSSG KFPWPRFLFF FLTLKTGGSG LNIKPTISAI AQMMNPMTIT
 EMNNQMRLE QKLLLFLPGS LFLRLSTILH YPGEGSNRPD PLEHALRRSR
 SLGLDQEEAA KKVIKVRGRDS KNDYVNVVEN QAASFLRRCG PSKRIQSVNY
 CKSTRQGHEI PDVKPLFPTG GGTQAPSRSR ARYAVPAILL GFAGFVGFLH
 YNDERRAVPR GQASSNSGCG CGSNTTVKGP IIGGPFTLVS TENKIVTEND
 40 FCGKWVLLYF GYSFSPDVGP EQLKMMMSKAV DKLAILLNPL TFGCLYLYAE
 FDSRILGLTG TASAMRQMAQ EYRVYFKKVQ EDGEDYLVDT SHNMYLINPK
 MEIVRCFGVE YNPDELSQEL LKEVASVSQ*

At1g32270 syntaxin, putative

45 MVRSNDVKFQ VYDAELTHFD LESNNNLQYS LSLNLSIRNS KSSIGIHYDR
 FEATVYYMNQ RLGAIVPMPLF YLGSKNTMLL RALFEGQTLV LLKGNERKKF
 EDDQKTGVYR IDVKLISINFR VMVLHLVTWP MKPVVRCHLK IPLALGSSNS
 TGGHKKMLLI GQLVKDTSAN LREASETSDHR RDVAQSKKIA DAKLAKDFEA
 ALKEFQKAQH ITVERETSYI PFDPKGFSSE SEVDIGYDRS QEQRVLMESR
 50 RQEIVLILDNE ISLNEARIEA REQGIQEVKH QISEVMEMFK DLAVMVDHQG
 TIDDIDEKID NLRSAAAQGK SHLVKASNTQ GSNSSSLFSC SLLLFFFLSG
 DLCRCVCVGS ENPRLNPTRR KAWCEEDEE QRKKQQKKKT MSEKRRREEK
 KVNKPNGFVF CVLGHK*

55

At1g13050

MSHHHYETNP HFVQFSLQDQ HQGGPSSSWN SPHHHQIPQA HSVAPPRVKI KTRGRHQTEP
 PETIHEPSS RPLPLRPEEP LPPRHNPNSA RPLQLSPEEQ RPPHRYGSE PTPWRRAPTR
 PAYQQGPKRT KPMTLPATIC CAILLIVLIL SGLLLLLVYL ANRPRSPYFD ISAATLNTAN
 5 LDMGYVLNGD LAVVNVFTNP SKKSSVDFSY VMFELYFYNT LIATEHIEPF IVPKGMSMFT
 SFHLVSSQVQ IQMIQSQDLQ LQLGTGPVLL NLRGTFHARS NLGSLMRYSY WLHTQCSISL
 NTPPAGTMRA RRCNTKR*

At5g45320

10 MPRPLTSRHGT SPFIWCAAI CAIISIVVIV GGIIVFVGYL VIHPRVPIIS
 VADAHLDFLK YDIVGVLQTO LTIVIRVEND NAKAHALFDE TEFKLSYEGK
 PIAILKAPEF EVVKEKSMFL PYLVQSYPIP LNPTMMQAVD YAVKKDVITF
 ELKGGSRTRW RVGPLGSVKF ECNLSCQLRF RPSDHSYIPS PCTSAHKH*

At3g20610

15 MDRDDAWEWF VTIVGSLMTL LYVSFLLALC LWLSTLVHHI PRCSIHYFYI PALNKS LISS
 DNTTLNFMVR LKNINAKQGI YYEDLHLSFS TRINNSSLV ANYTVPRFYQ GHEKKAKKWG
 QALPFNNQTV IQAVLPNGSA IFRVDLKMQV KYKVMWSWTK RYKLKASVNL EVNEDGATKV
 KDKEDGIKMK ISDSSPQRLLT FFQVCFSIIC VLMNWLIPLA IR*

At4g26490

20 MVLTKPATVR FNGLDAEPRK DRVILRQPRS SRTSLWIWCV AVFLAIRPRI PVFDIPNANL
 HTIYFDTPEF FNGLDLSMLVN FTNPNNKIEV KFEKLRIELF FFNRLIAAQV VQPFLQKKHE
 TRLEPIRLIS SLVGLPVNHA VELRQLENN KIEYEIRGTF KVKAHFGMIH YSYQLHGRCQ
 LQMTGPPTGI LISRNCTTKK *

At5g42860

25 MHAKTDSEVT SLSASSPTRS PRRPAYFVQS PSRDSDHDGEK TATSFHSTPV
 LTSPMGSPPH SHSSSSRFPSK INGSKRKGHA GEKQFAMIEE EGLLDDGDRE
 QEA LP RRCYV LAFIVGFSLL FAFFSLILYA AAKPQKPKIS VKSITFEQLK
 30 VQAGQDAGGI GTDMITMNAT LRMLYRNTGT FFGVHVTSSP IDLSFSQITI
 GSGSIKKFYQ SRKSQRTVVV NVLGDKIPLY GSGSTLVPPP PPAPIPKPKK
 KKGPIVIVEP PAPPAPVPMR LNFTVRSRAY VLGKLVQPKF YKRIVCLINF
 EHKKLSKHIP ITNNCTVTSI *

At1g45688

35 MHAKTDSEVT SLAASSPARS PRRPVYYVQS PSRDSDHDGEK TATSFHSTPV LSPMGSPPHS
 HSSMGRHSRE SSSSRFSGSL KPGSRKVNPN DGSKRKGHGG EKQWKECAVI EEEGLLDDGD
 RDGGVPRRCY VLA FIVGFFI LFGFFSLILYA GAAKPMKPKI TVKSITFETL KIQAGQDAGG
 40 VGTDMITMNA TLRMLYRNTG TFFGVHVTST PIDLSFSQIK IGSGSVKKFY QGRKSERTVL
 VH VIGEKIPL YGSGSTLLPP APPAPLPKPK KKKGAPVPIP DPPAPPAPVP MTLSFVVRSR
 AYVLGKLVQP KFYKKIECDI NFEHKNLNKH IVITKNCTVT TV*

At4g26820

45 MDDEQNLVEE MNQQLLITVI DTEKVPRLP ISSRSHQESE PANISHWSLL FKLFLAITIM
 GACVAGVTIV ILITPTPPTV HVQSMHISFA NHNLPVWSAT FSIKNPNEKL HVTYENPSVW
 LVHRGKLVST ARADSFWQKG GEKNEVIVKR NETKVIDEEA AWEMEDEVAV TGGVVGLDMV
 FSGRVGFYPG TSALWGEQYM SAVCENSAK LYNVDDEIYG TNRSVLSFDG RLVCSVRLPK
 YP*

50

Plants respond in a variety of ways to pathogens. After a recognition of the pathogen, normally mediated by avr and R genes, the resulting response induces a hypersensitive

response, that results in inhibition of the pathogen. After the recognition, further processes appear to be non-specific.

In addition to the hypersensitive response, a second line of defence, defined as the systemic acquired resistance response

5 can be triggered, that renders unaffected parts of the plant resistant to a variety of normally virulent pathogens. Several of the RKS and ELS gene products prove to be key regulators in the regulation of the system acquired resistance response.

10 Overexpression of several of the RKS and / or ELS genes in plants, either by constitutive promoters, stage and / or tissue specific promoters, or inducible promoters allows the activation of a systemic acquired resistance response in plants.

15 Another application can be provided by the activation of a RKS /ELS specific ligand in (transgenic) plants, thereby activating the receptor complex, that finally results in triggered activation of the systemic acquired resistance response in these plants.

20 (ref. Generation of broad-spectrum disease resistance by overexpression of an essential regulatory gene in systemic acquired resistance. H. Cao et al. 1998. Proc. Natl. Acad. Sci. USA 95: 6531-6536). Recent literature shows the functional interaction between RKS10 and BRI-1, another class 25 of transmembrane LRR receptor kinases (Cell Vol. 110, 213-222 2002). BAK1=RKS10 as described here, interacts with BRI-1 and modulates brassinosteroid signaling; Cell vol 110, 203-212 2002 BRI1/BAK1 a receptor kinase pair mediating brassinosteroid signaling). Brassinosteroids are known to 30 function in a broad range of disease resistance in tobacco and rice (Plant Journal 2003, 887-898). The BRI-1 receptor is involved in the binding of systemin, an 18 amino acid polypeptide, representing the primary signal for the systemic activation of defence genes (PNAS 2002, 9585-9590). 35 ELS overexpression phenotypes mimic the effects of inactivation of RKS molecules gene products. Either ELS is competing for ligand binding, or ELS inhibits the interactions

between RKS and BRI-1-like gene products. ELS1 overexpression results in dwarf phenotypes in *Arabidopsis* and tobacco plants, similar as observed for antisense RKS4 and RKS10, and for knock out plants of RKS0 and RKS4.

5 Deregulating expression of ELS and / or RKS genes in plant would modify the broad spectrum disease resistance in such plants. This would explain the observed data that brassinosteroids are involved in disease resistance (Plant Journal 2003, 33 887-898.)

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Claims

1. A method for modulating a developmental pathway of a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signaling complex comprising RKS protein, ELS protein, NDR/NHL protein, SBP/SPL protein and RKS/ELS ligand protein or encoding a protein comprising a ligand for said complex.

10

2. A method according to claim 1 allowing modulating cellular division during plant growth or organ formation

3. A method according to claim 2 wherein said gene comprises

15 an RKS4 or RKS10 gene or functional equivalent thereof.

4. A method according to claim 1 allowing modulating apical meristem formation.

20 5. A method according to claim 4 wherein said gene comprises an ELS1, RKS0, RKS3, RKS4, RKS8 or RKS10 gene or functional equivalent thereof.

6. A method according to claim 4 allowing modulating

25 fasciation.

7. A method according to claim 6 wherein said gene comprises an RKS0, RKS3, RKS8 or RKS10 gene or functional equivalent thereof.

30

8. A method according to claim 4 allowing modulating root development.

35 9. A method according to claim 7 wherein said gene comprises an ELS1, ELS 2, RKS1, RKS3, RKS4, RKS6, RKS8 or RKS10 gene or functional equivalent thereof.

10. A method according to claim 4 allowing modulating meristem identity.

11. A method according to claim 9 wherein said gene comprises 5 an ELS1, RKS8, RKS10 or RKS13 gene or functional equivalent thereof.

12. A method according to claim 1 allowing modulating pollen development.

10

13. A method according to claim 11 wherein said gene comprises an ELS2 or RKS10 gene or functional equivalent thereof.

15

14. A method for providing resistance to a plant or plant cell comprising modifying a gene or modifying expression of said gene, wherein said gene is encoding a protein belonging to a signalling complex comprising NDR/NHL protein, or encoding a protein comprising a ligand for said complex.

20

15. A method for obtaining a plant or plant cell with a modulated development comprising subjecting a plant or plant cell to a method according to anyone of claims 1 to 13.

25

16. A method for obtaining a resistant plant or plant cell comprising subjecting a plant or plant cell to a method according to claim 14.

30

17. A plant or plant cell obtainable with a method according to claim 15 or 16.

Fig. 1

Different domains of RKS proteins

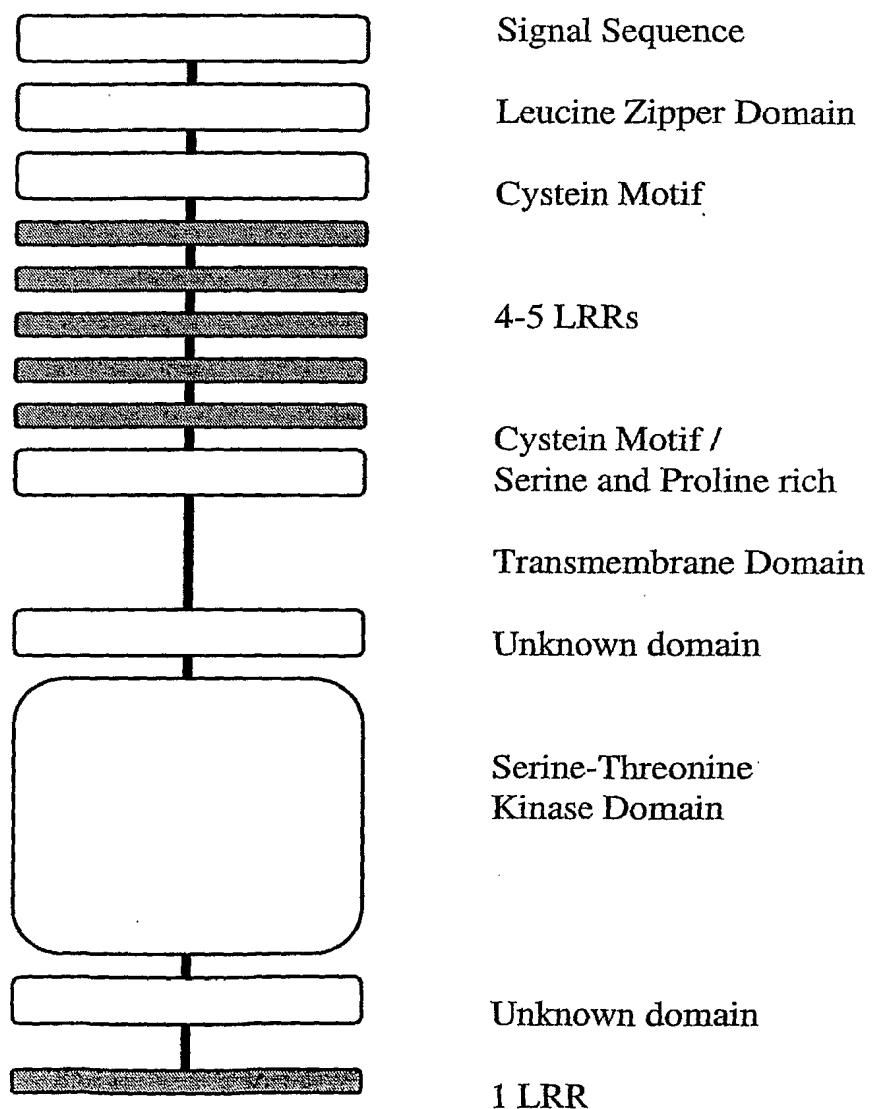


Fig. 2

Developmental tree of the different Receptor Kinases like SERK (RKS) genes.

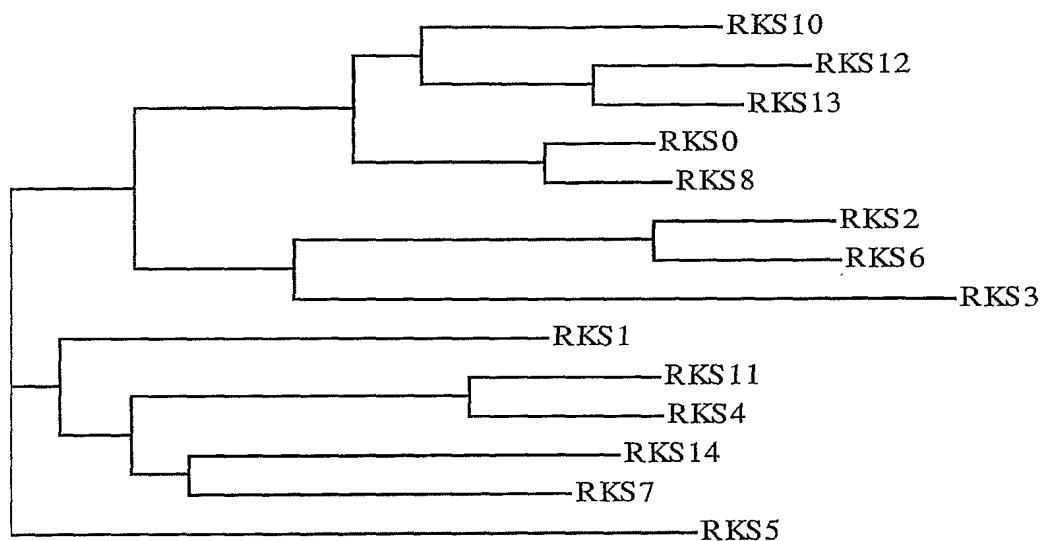


Fig. 3

Intron-Exon structure of the RKS genes in *Arabidopsis thaliana* var. Columbia.
SS signal sequence; LRR leucine rich repeat domain; TM transmembrane domain.

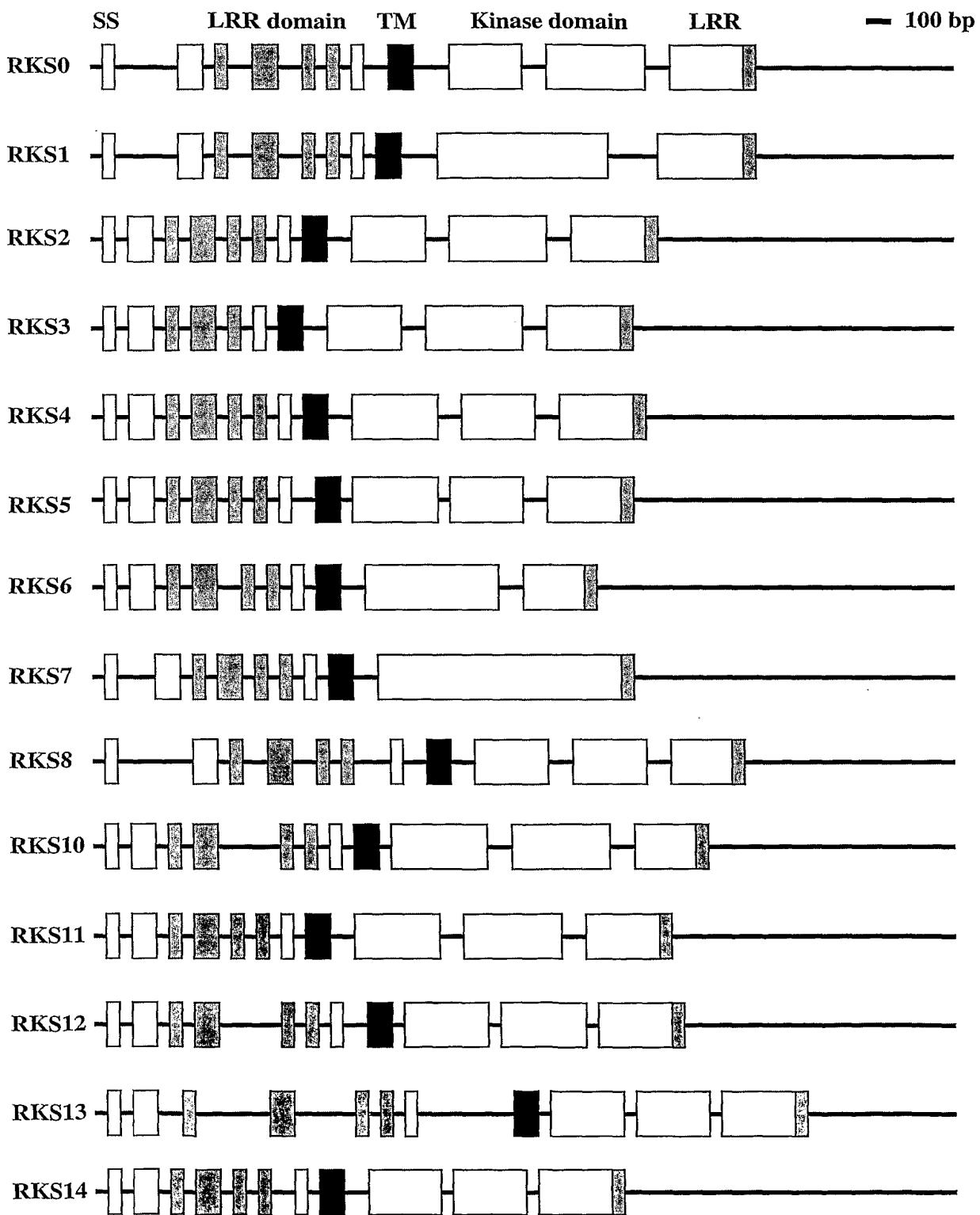
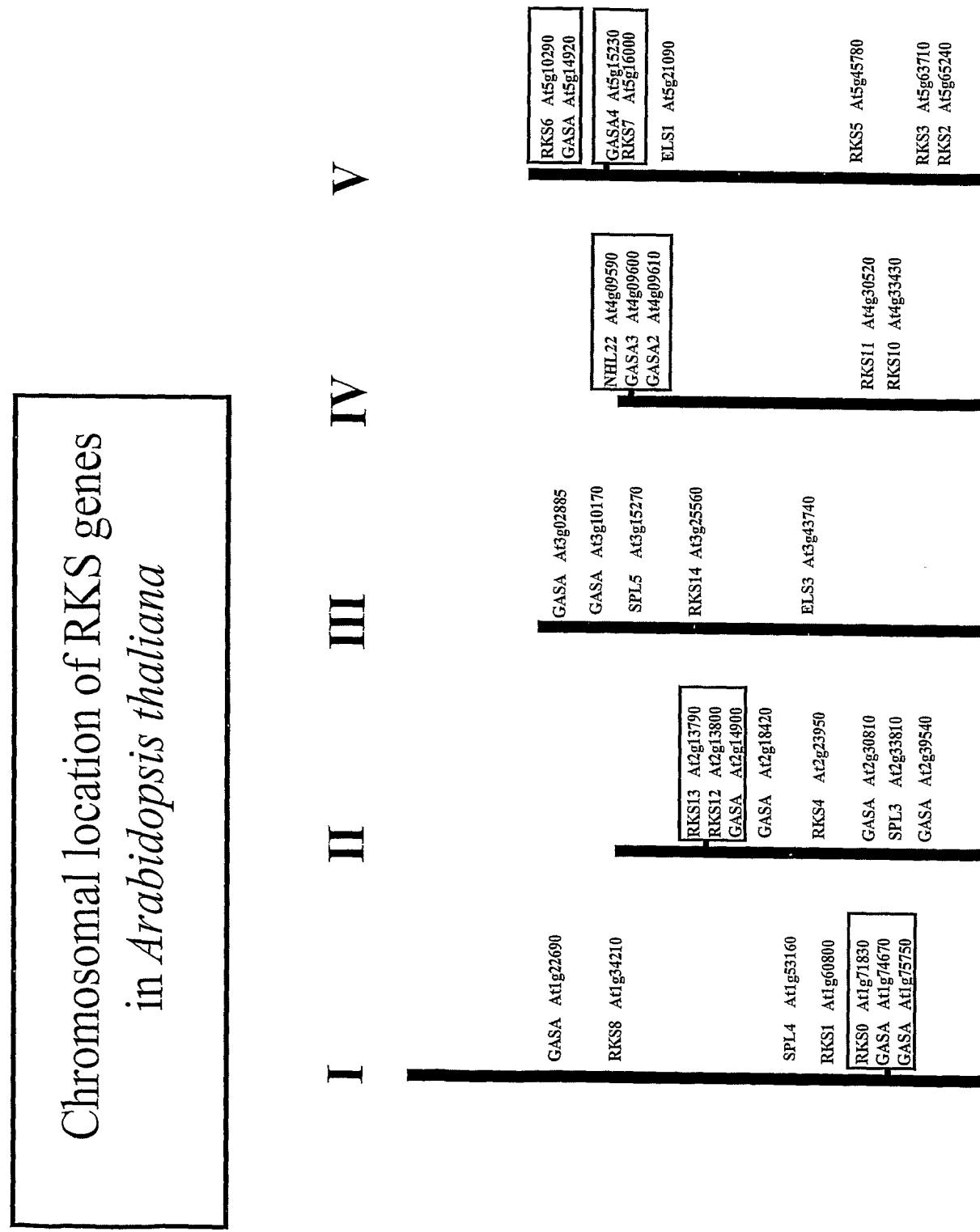
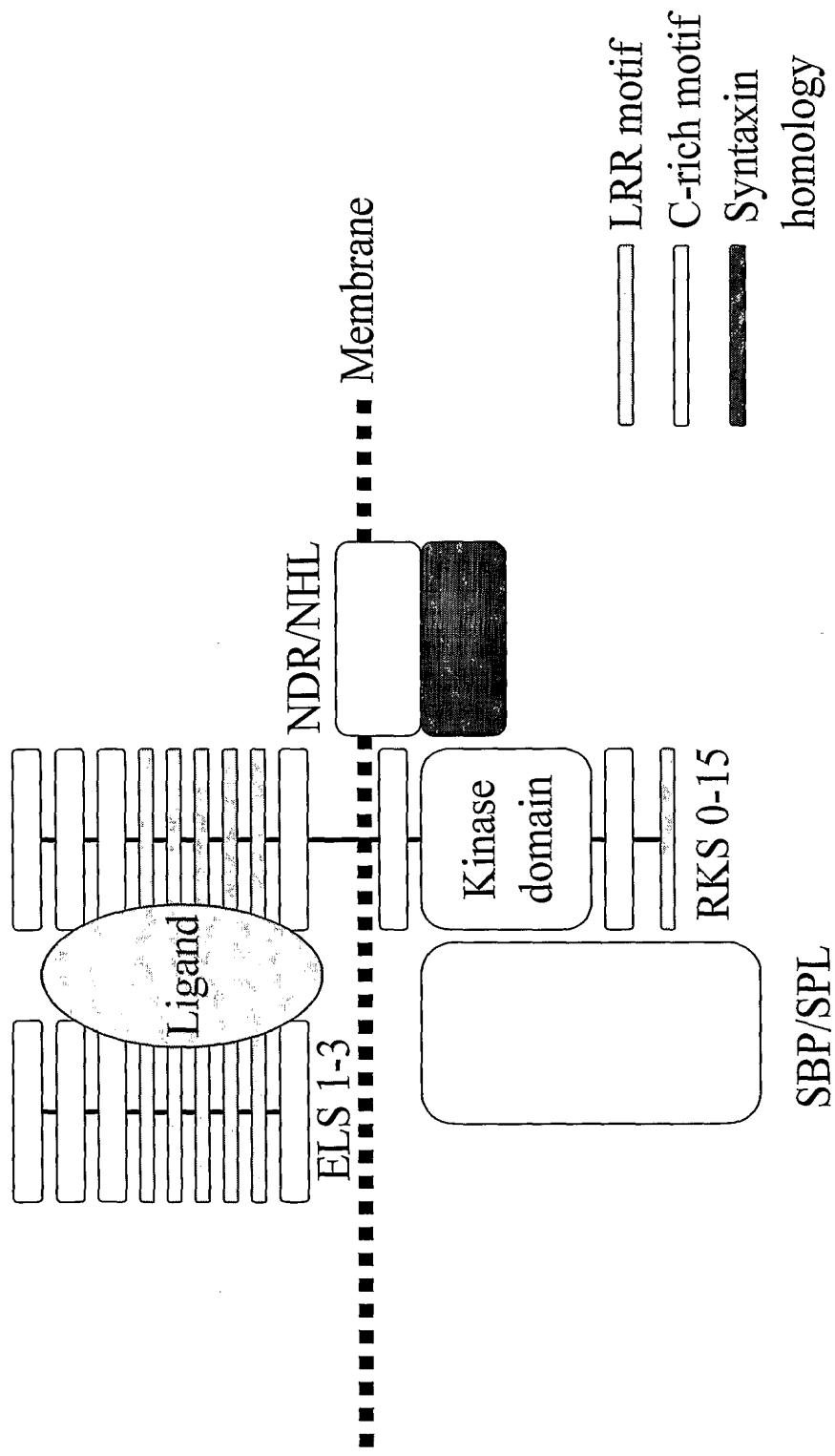


Fig. 4



RK5-mediated signal transduction pathway in plants

Fig. 5



GT-RKS4 determines seedling size
in *Nicotiana tabacum*.

Modifications in the
expression profile
of GT-RKS4 modulates
organ size within seedlings
of *Nicotiana tabacum*.

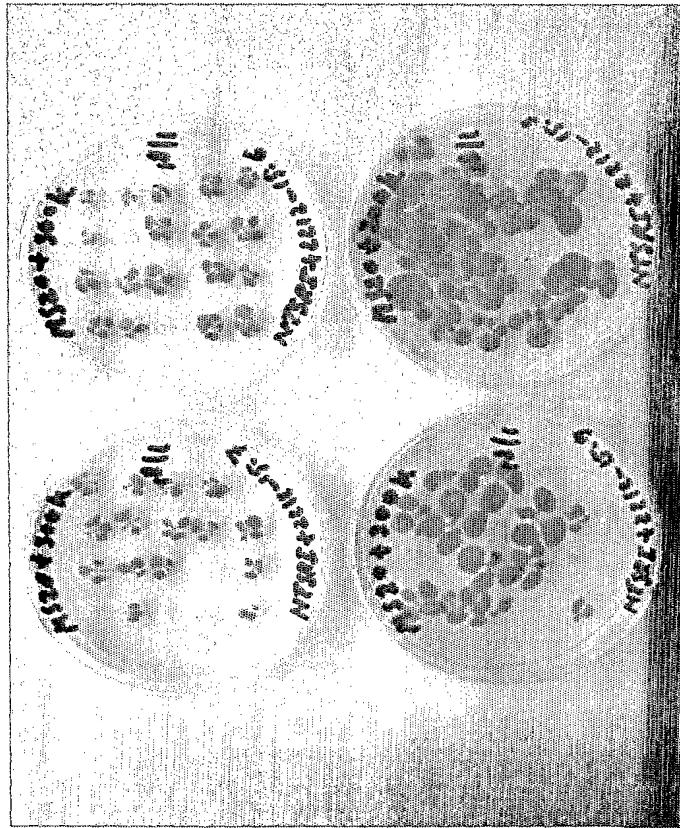


Fig. 7

GT-RKS4-7S-T2

GT-RKS4-6S-T2

GT-RKS4-3S-T2

GT-RKS4 determines organ size
in *Nicotiana tabacum*.

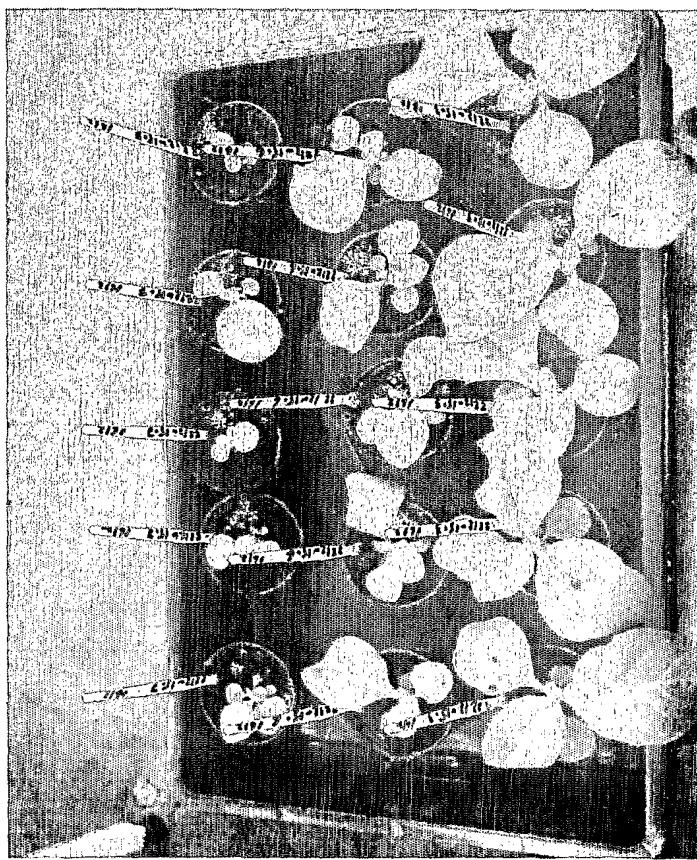
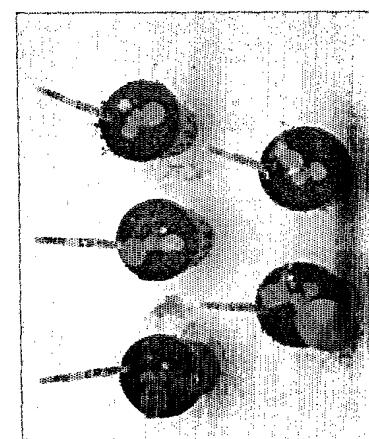
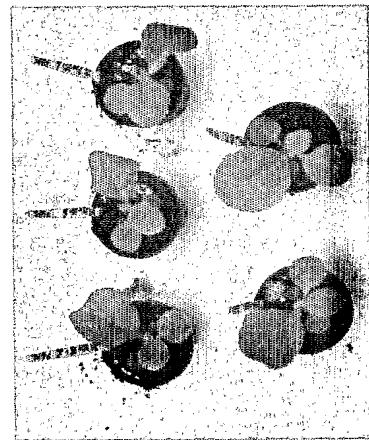
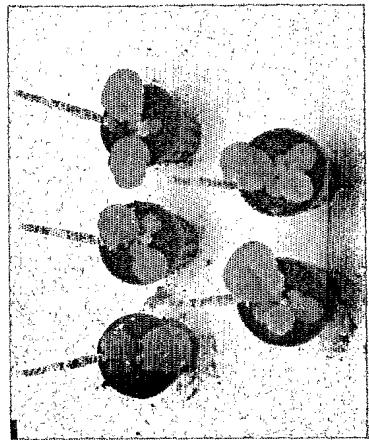
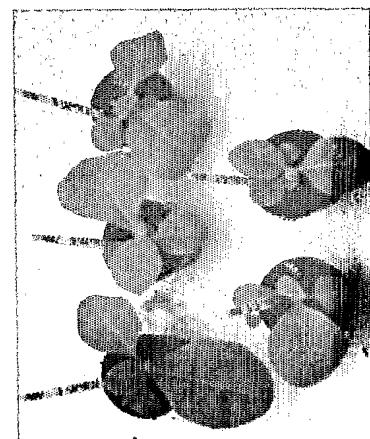
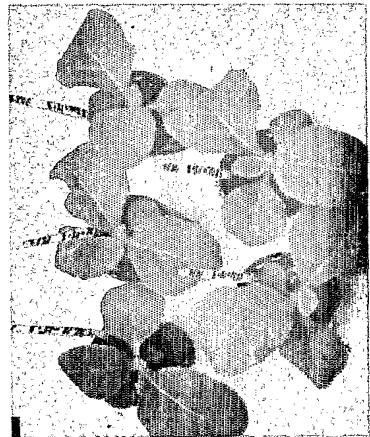


Fig. 8

GT-RKS4 determines plant size
in *Nicotiana tabacum*



GT-RKS4-15S-7T2 GT-RKS4-15S-6T2 GT-RKS4-15S-3T2

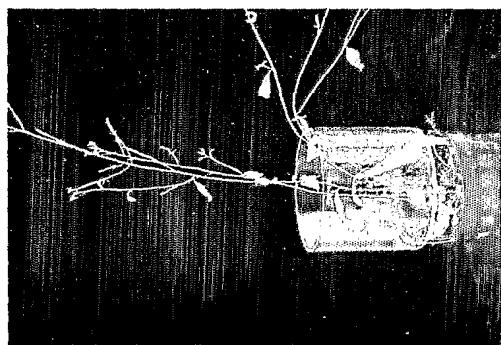
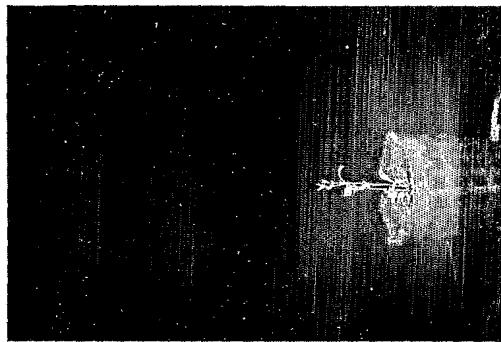


GT-RKS4-15S-9T2 GT-RKS4-15S-3T2

Fig. 9

Stable transformed GT-RKS4-antisense
in *Arabidopsis thaliana*

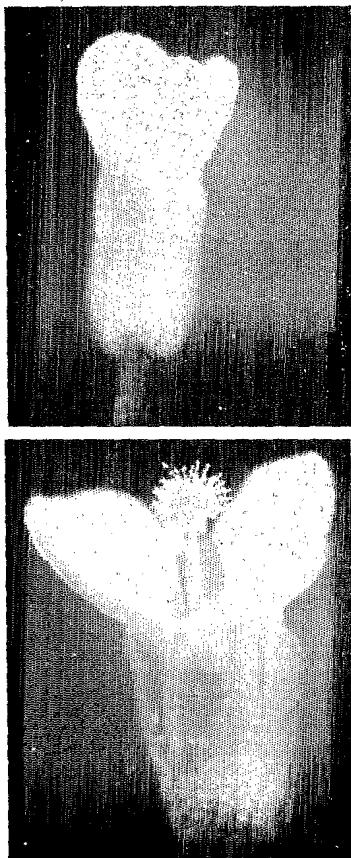
Wildtype WS GT-RKS4-16a



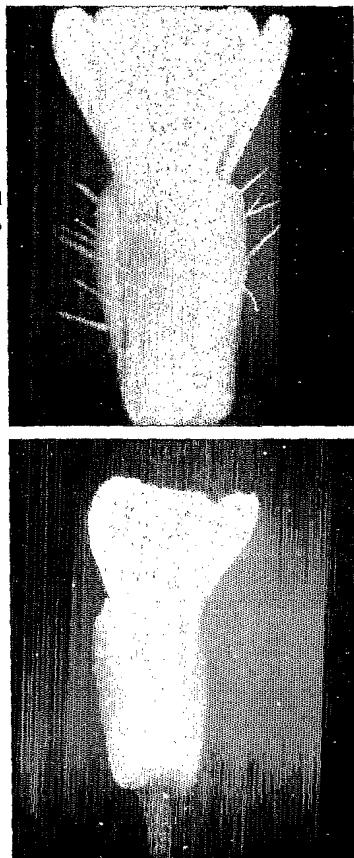
Overexpression of antisense GT-RKS4-1a
reduces plant and organ size.

Fig. 10

Ectopic expression of RKS4 and GASA3 gene products both result in increases flower size in *Arabidopsis thaliana* WS



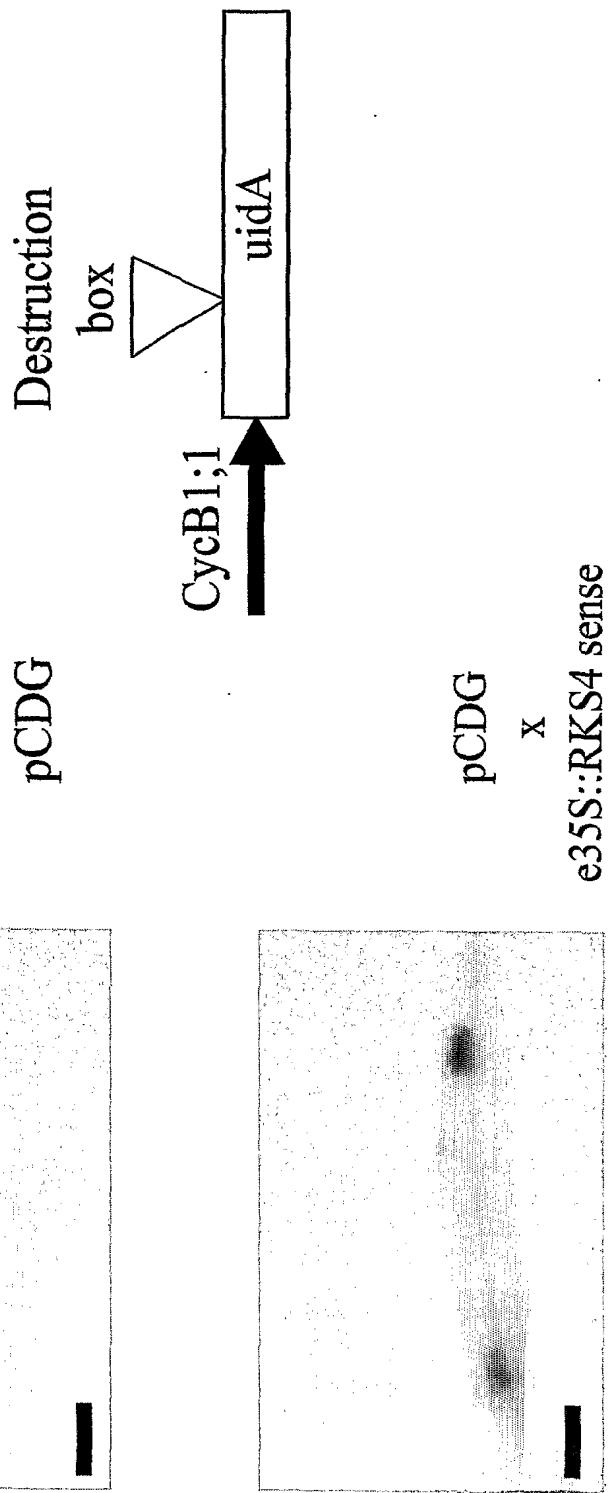
e35S::RKS4 sense



Wildtype WS

e35S::RKS4
antisense
e35S::GASA3 sense

Fig. 11



Ectopic expression of RKS4 in seedlings results in the formation of meristematic regions in the hypocotyl of *Arabidopsis thaliana* WS

Fig. 12

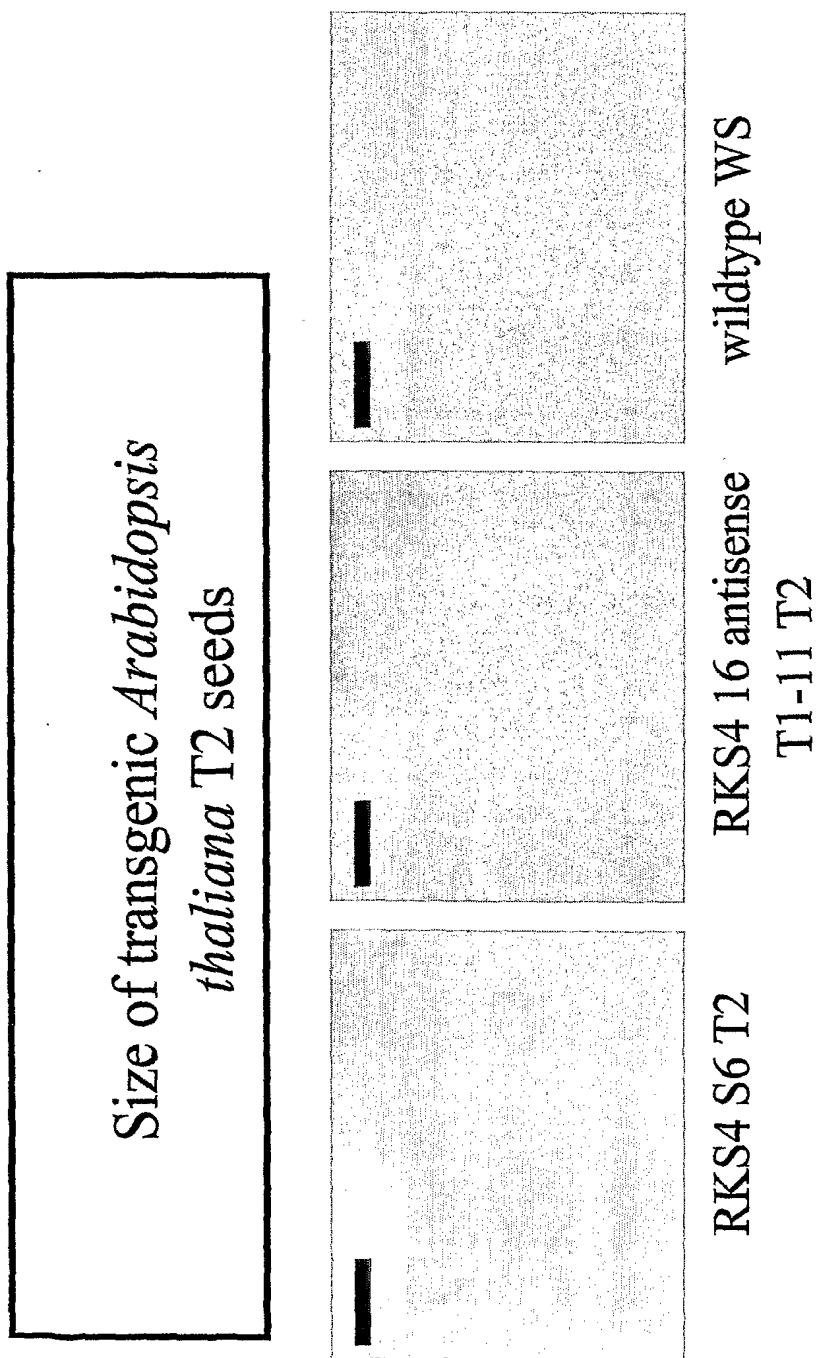


Fig. 13

RKS4 regulates cell number and cell size in *Arabidopsis thaliana*.

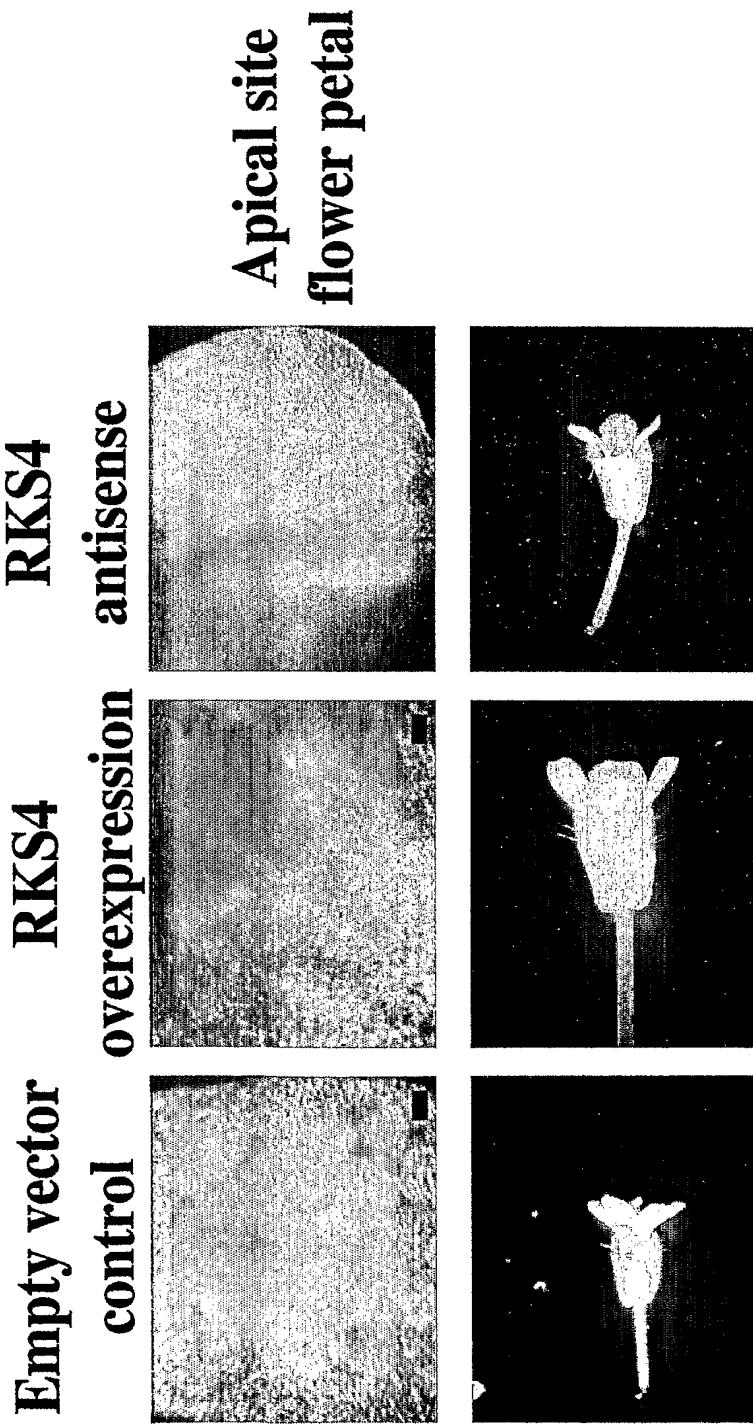
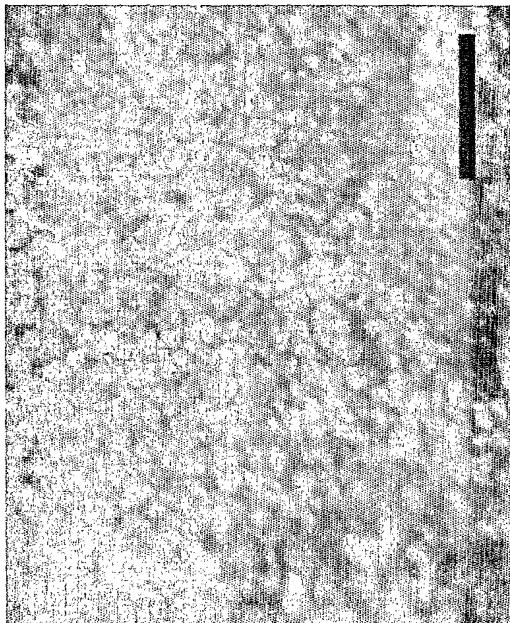
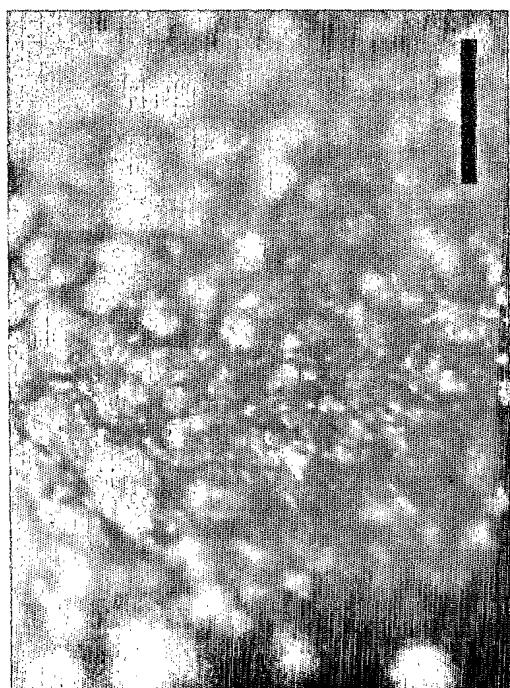


Fig. 14

RKS10S T1-10
results in a decrease in size
of cotyl-like apical epidermal cells



RKS10S T1-10



pGreen 4K

Fig. 15

RKS10 antisense T1-4
results in an increase in size
of the cotyl epidermal cells

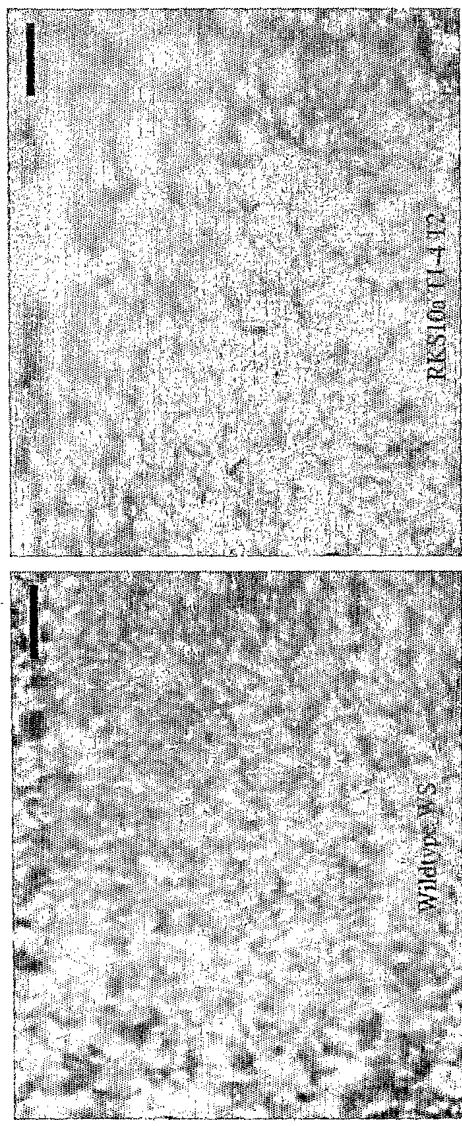


Fig. 16

Flower development from the same
influorescence in transgenic
Arabidopsis thaliana

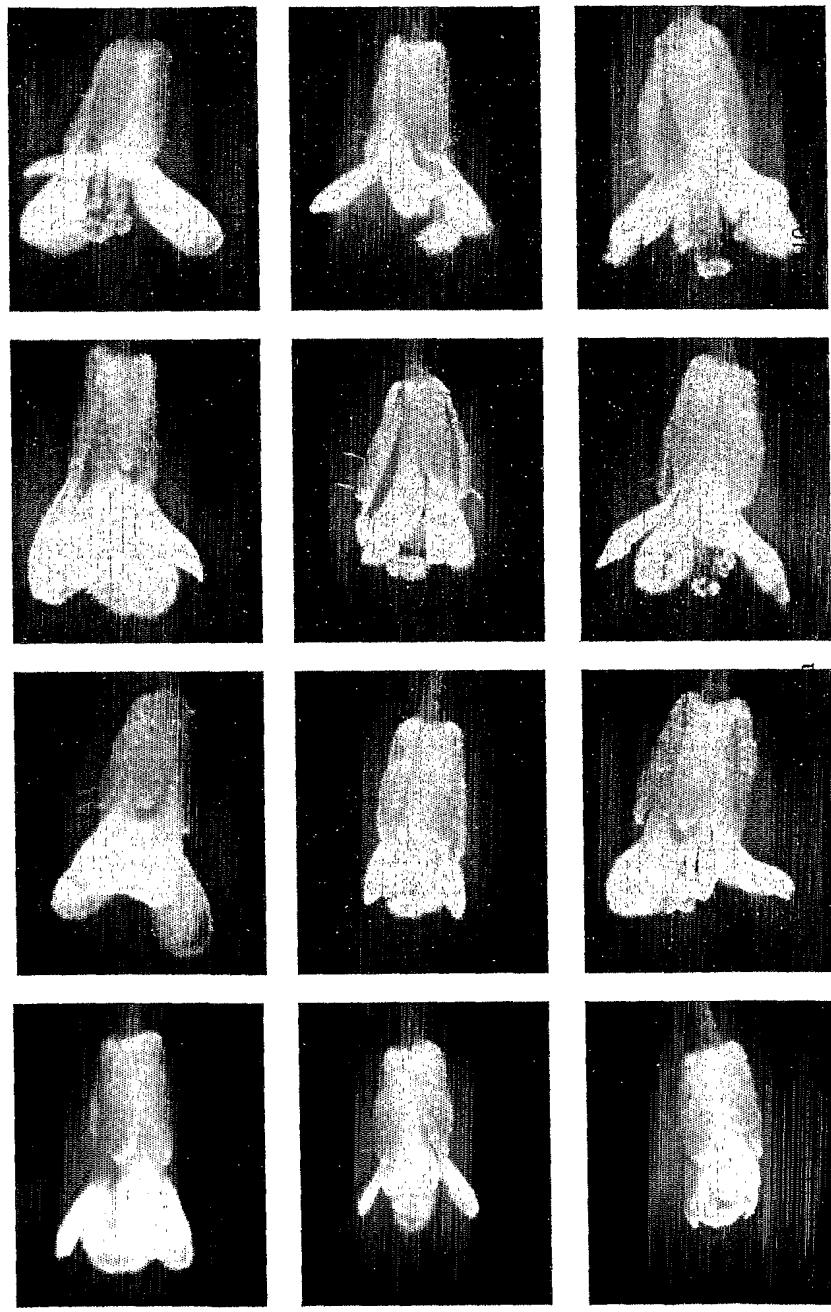


Fig. 17

Regeneration potential of
Arabidopsis transgenic seedlings.

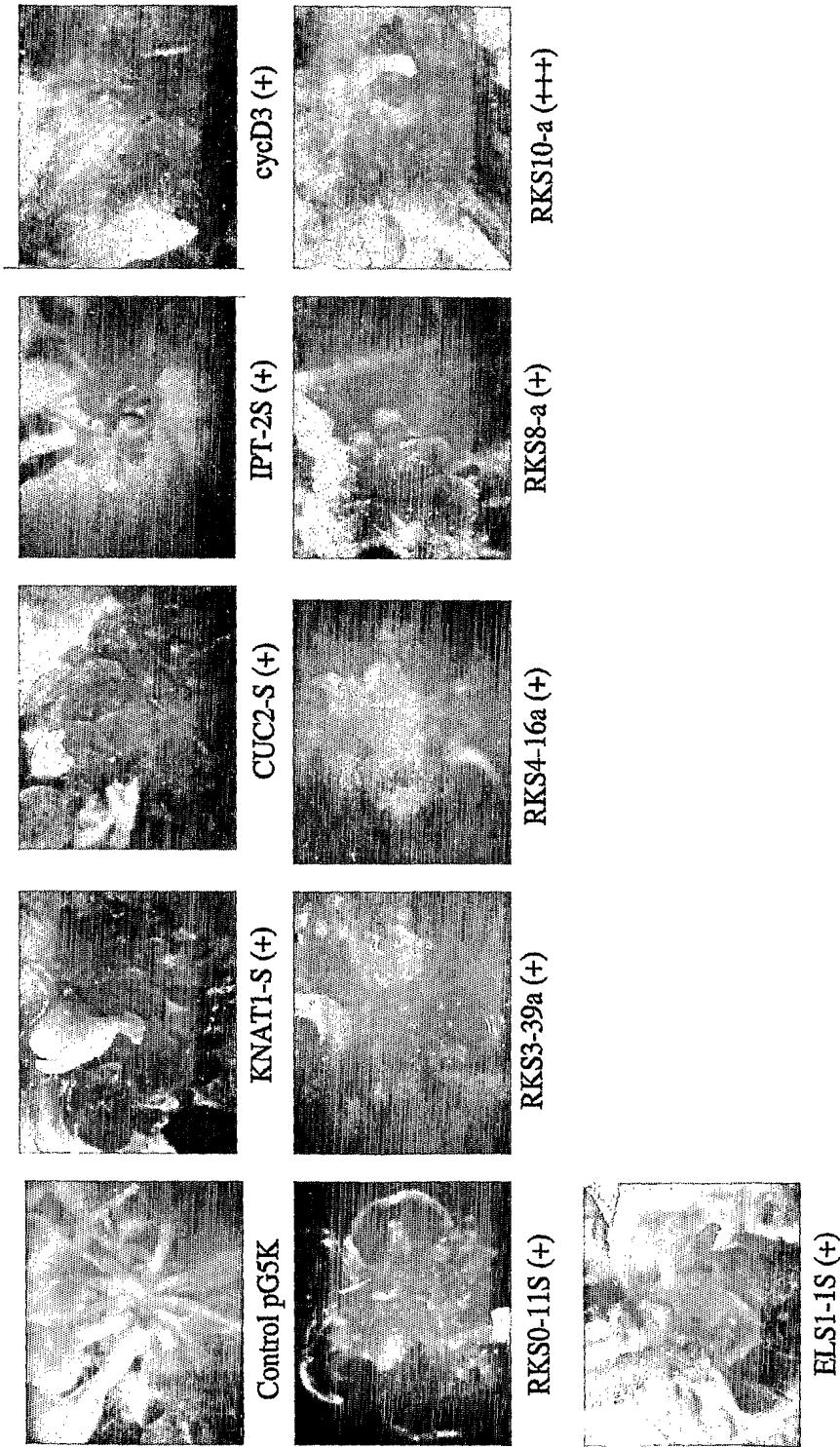


Fig. 18

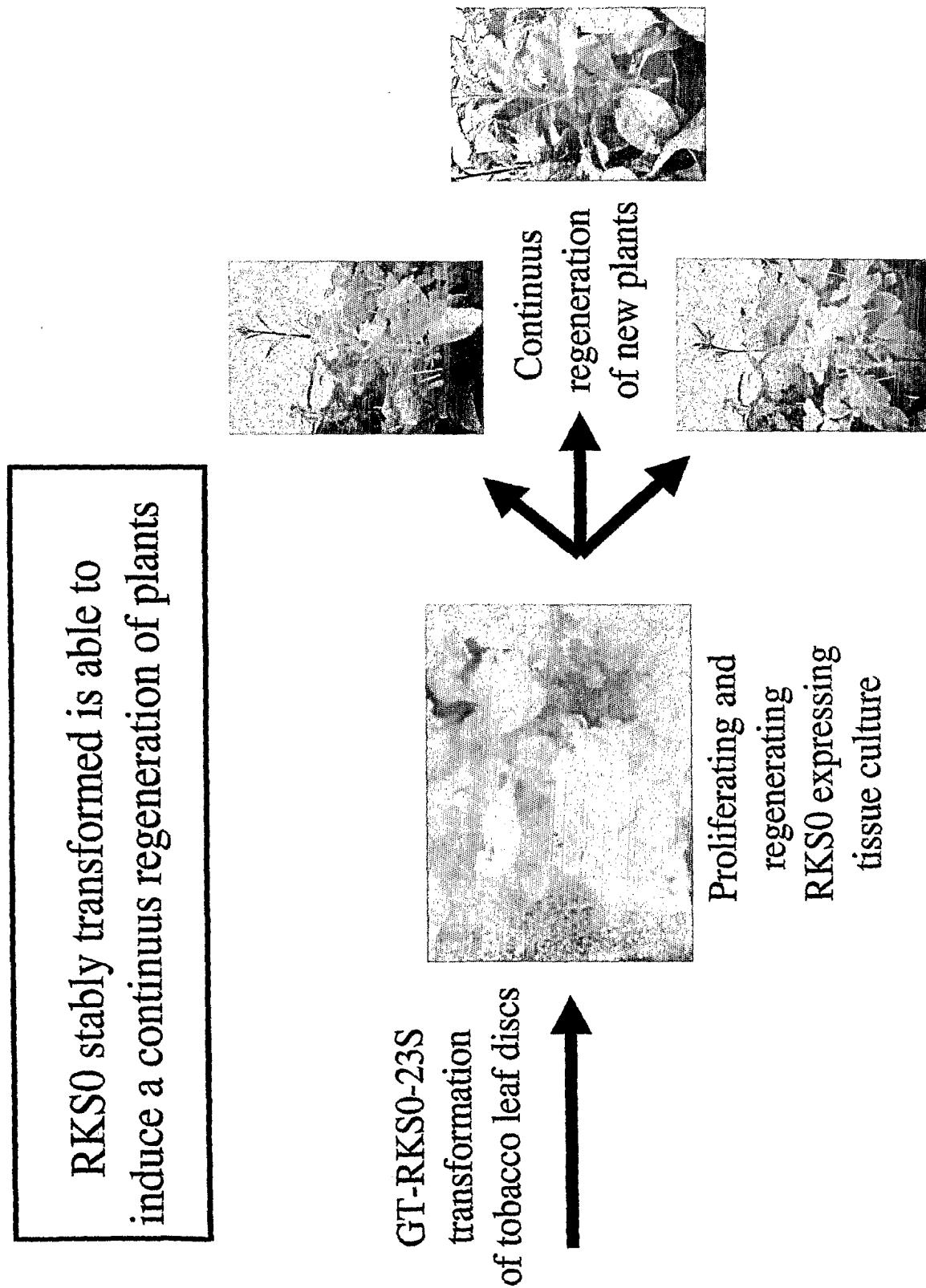


Fig. 19

Fasciation in transgenic
Arabidopsis thaliana

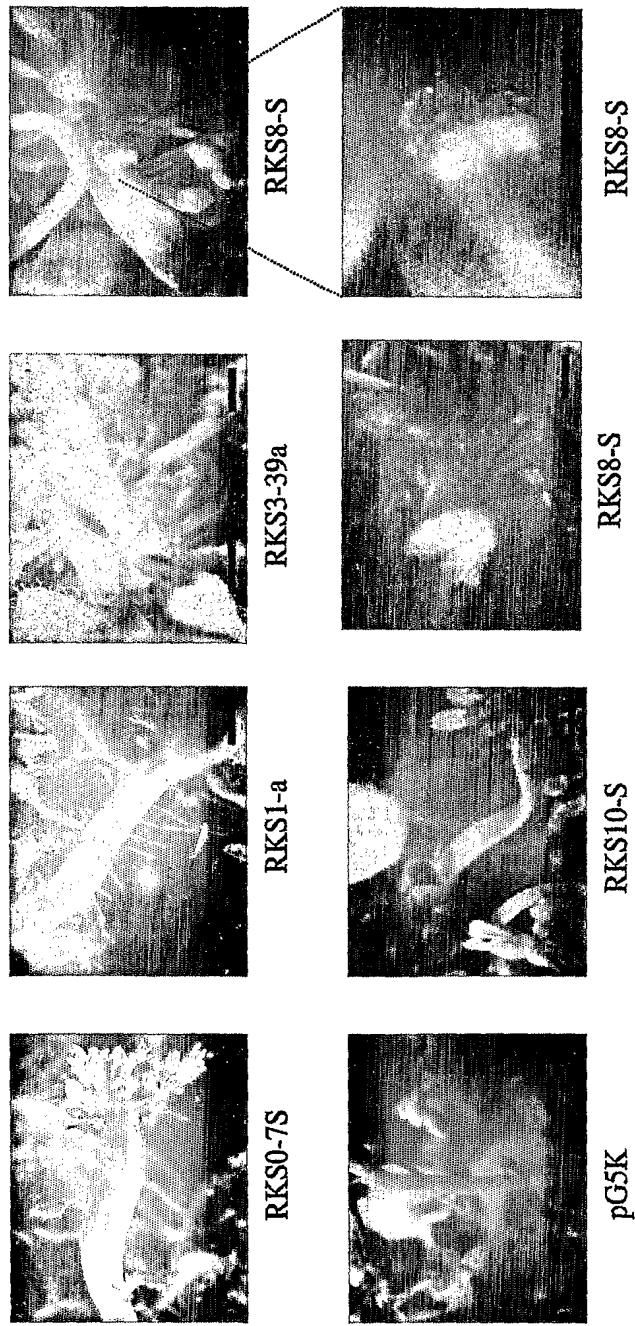


Fig. 20

Root growth of transgenic
Arabidopsis thaliana

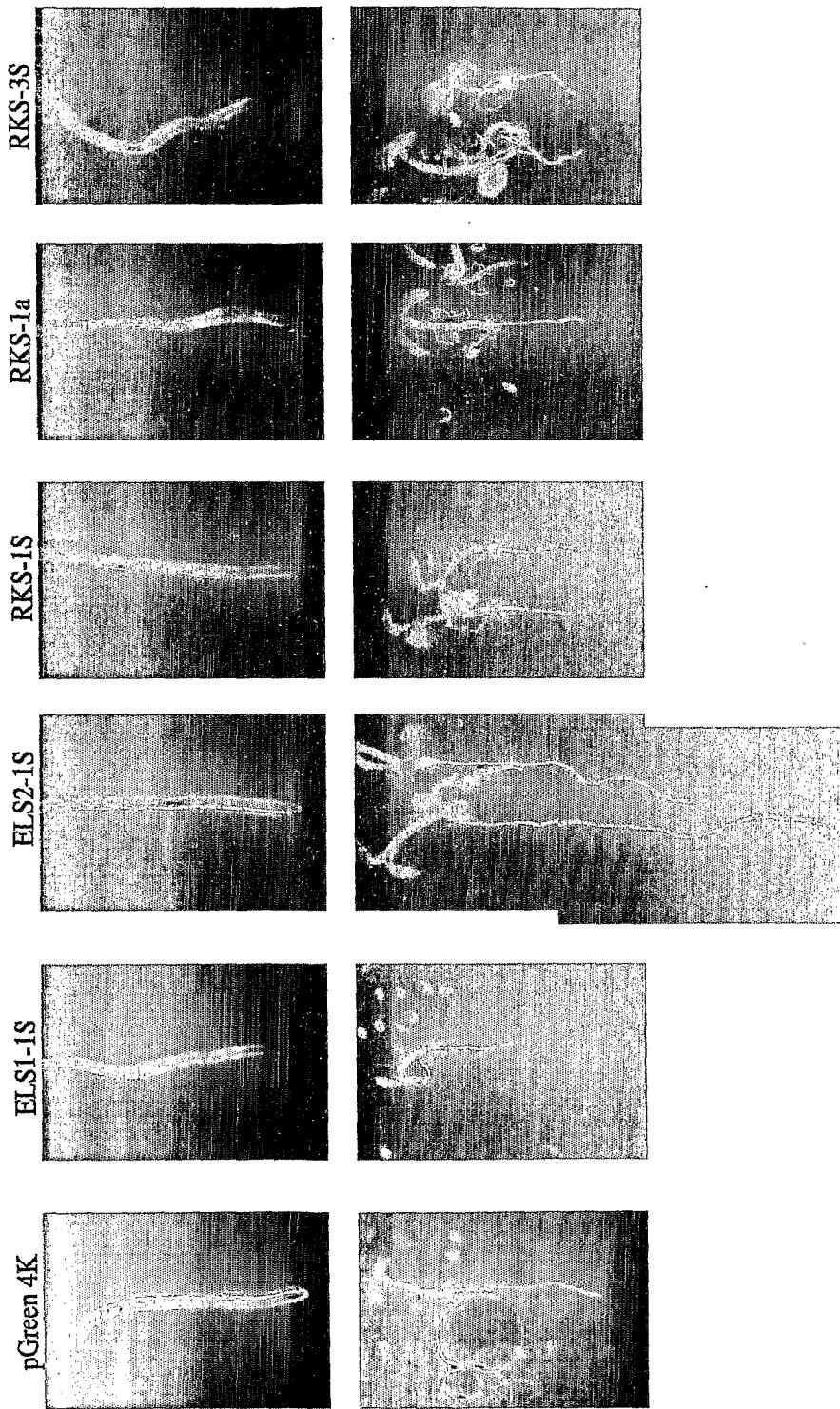


Fig. 21

Root growth of transgenic
Arabidopsis thaliana

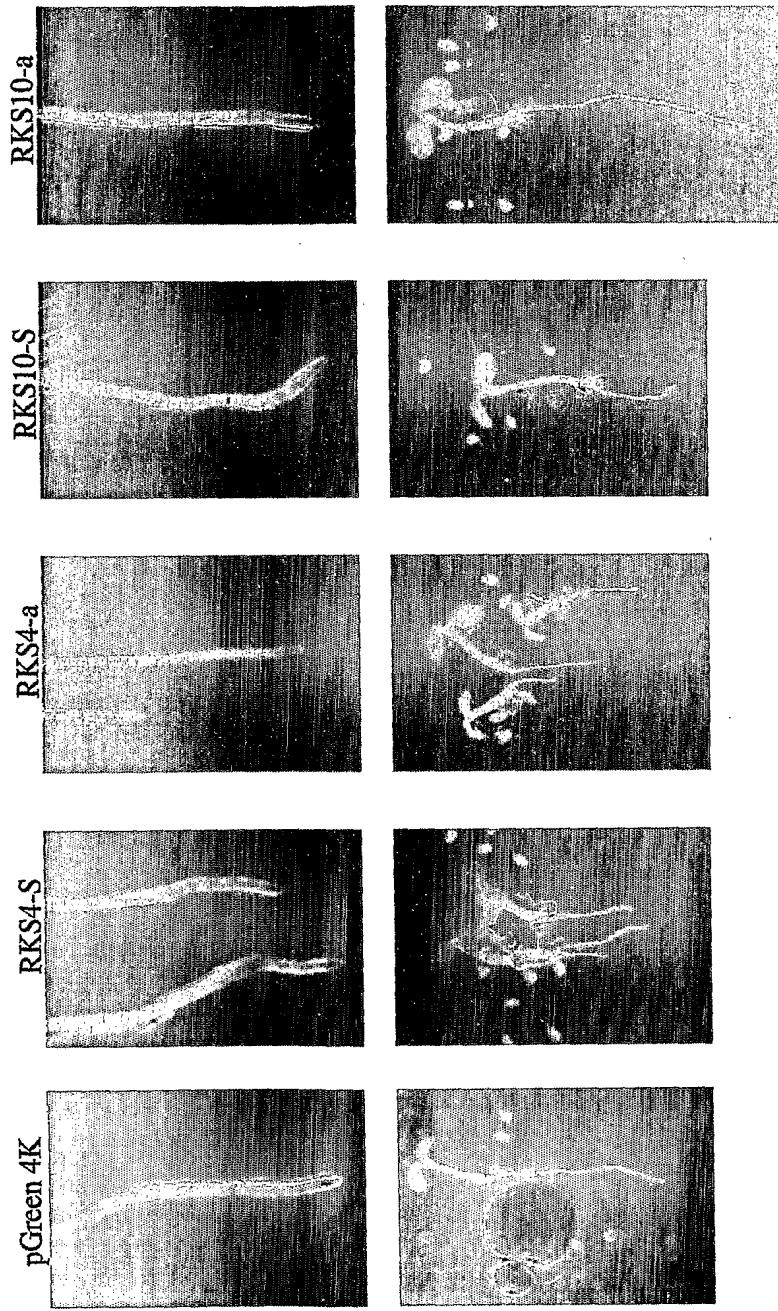


Fig. 22

Root growth of transgenic
Arabidopsis thaliana

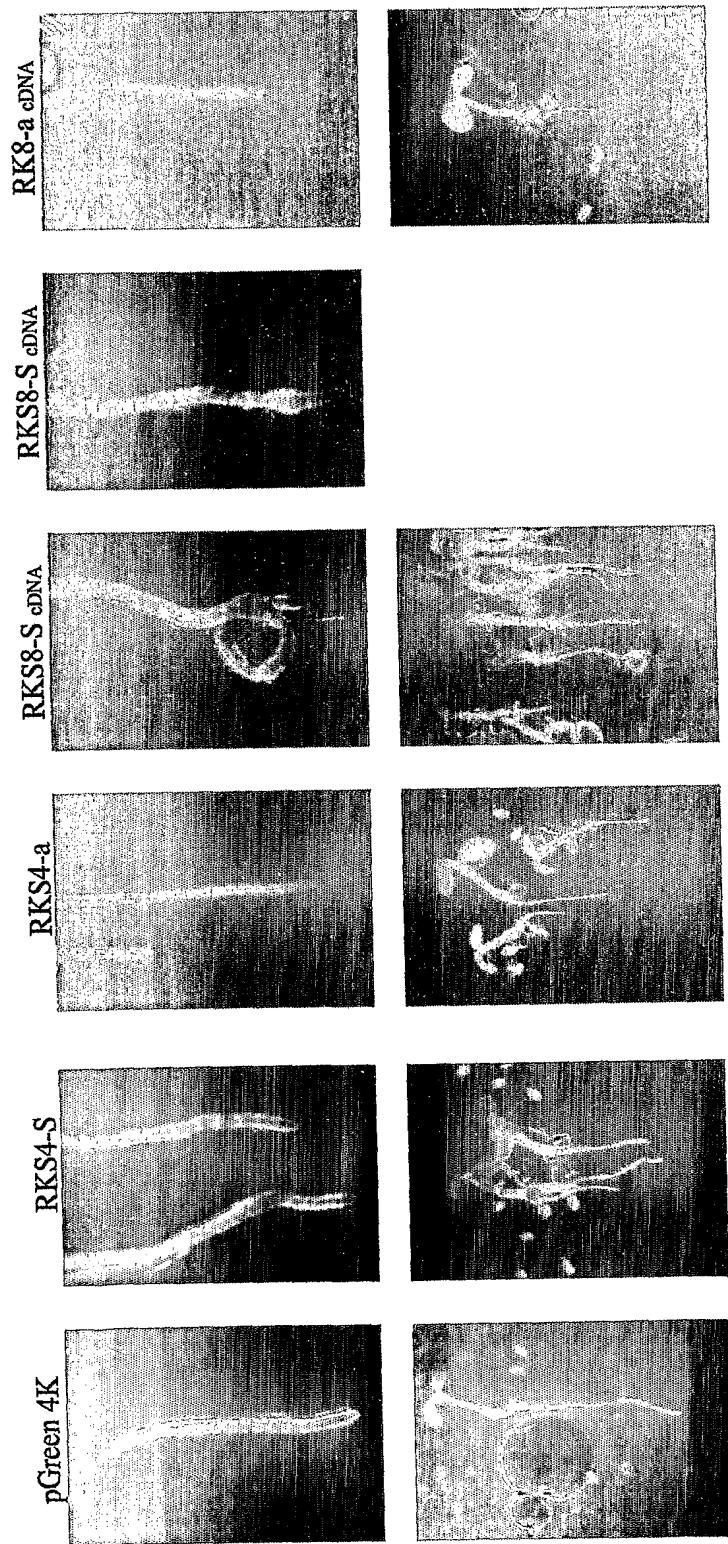


Fig. 23

Root growth of transgenic
Arabidopsis thaliana

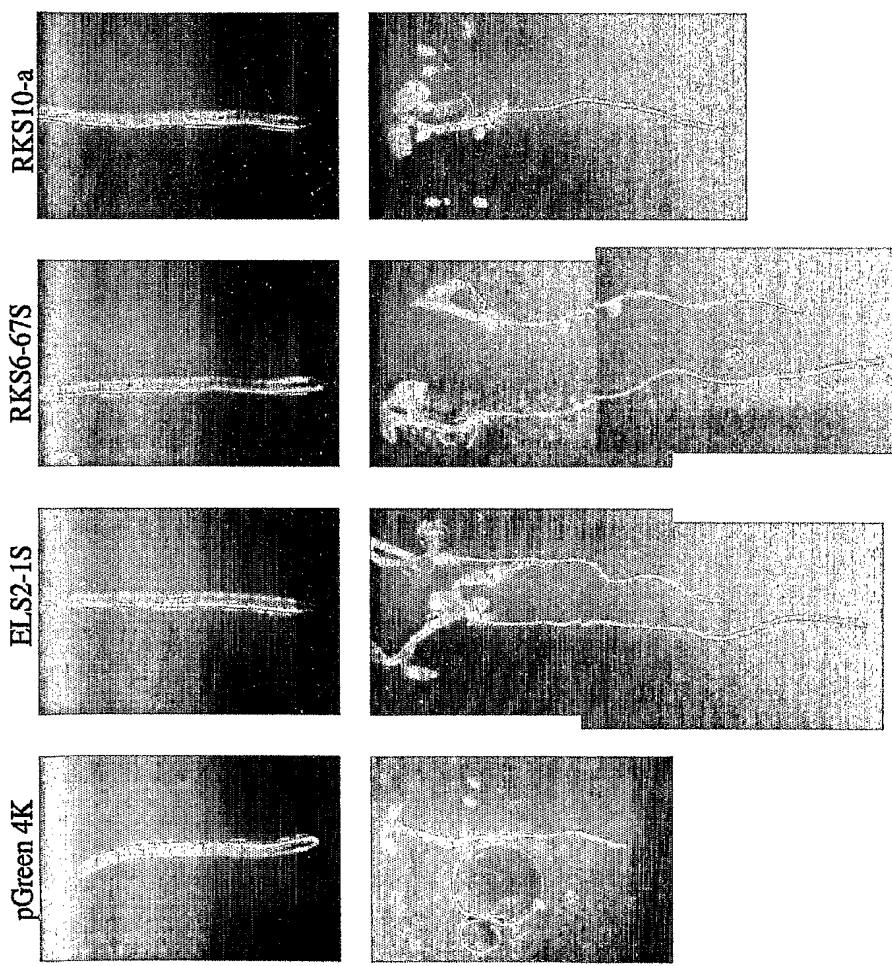
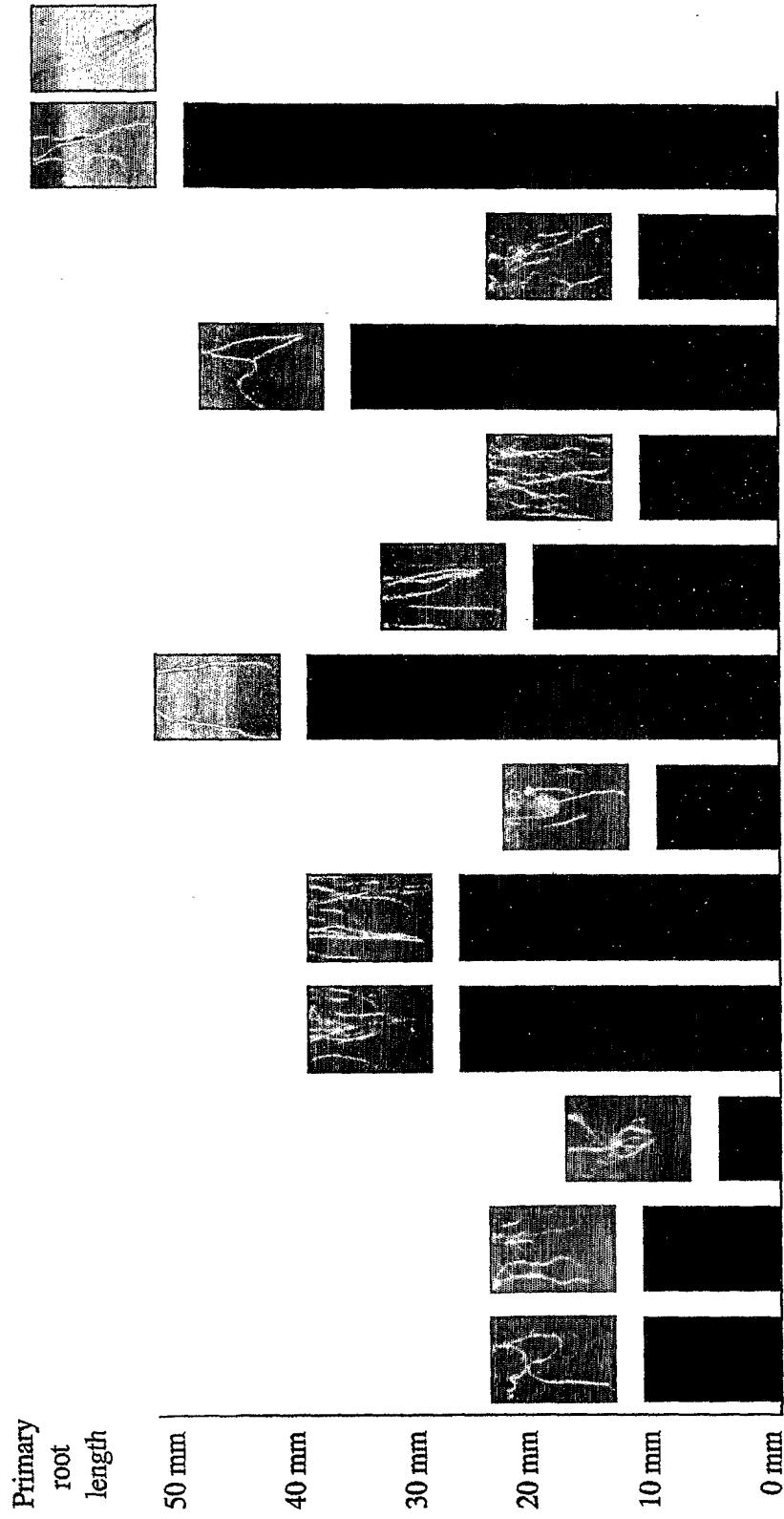
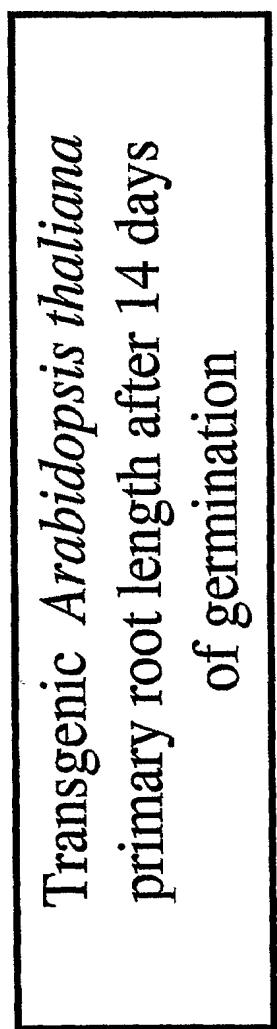


Fig. 24



Transgenic construct

Fig. 25

Effects of RKS10 transgenic constructs on plant development of 45 days old *Arabidopsis* WS

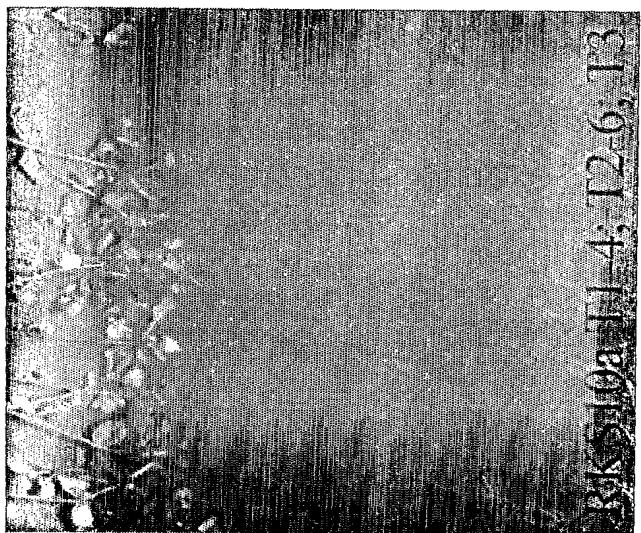
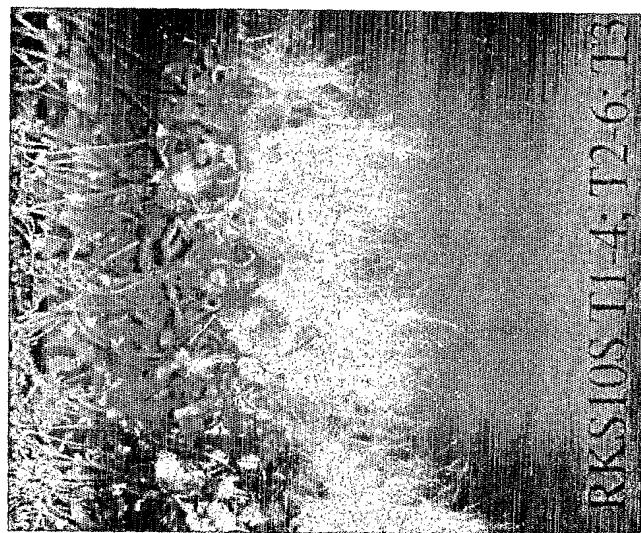


Fig. 26

Roots of Transgenic
Arabidopsis thaliana

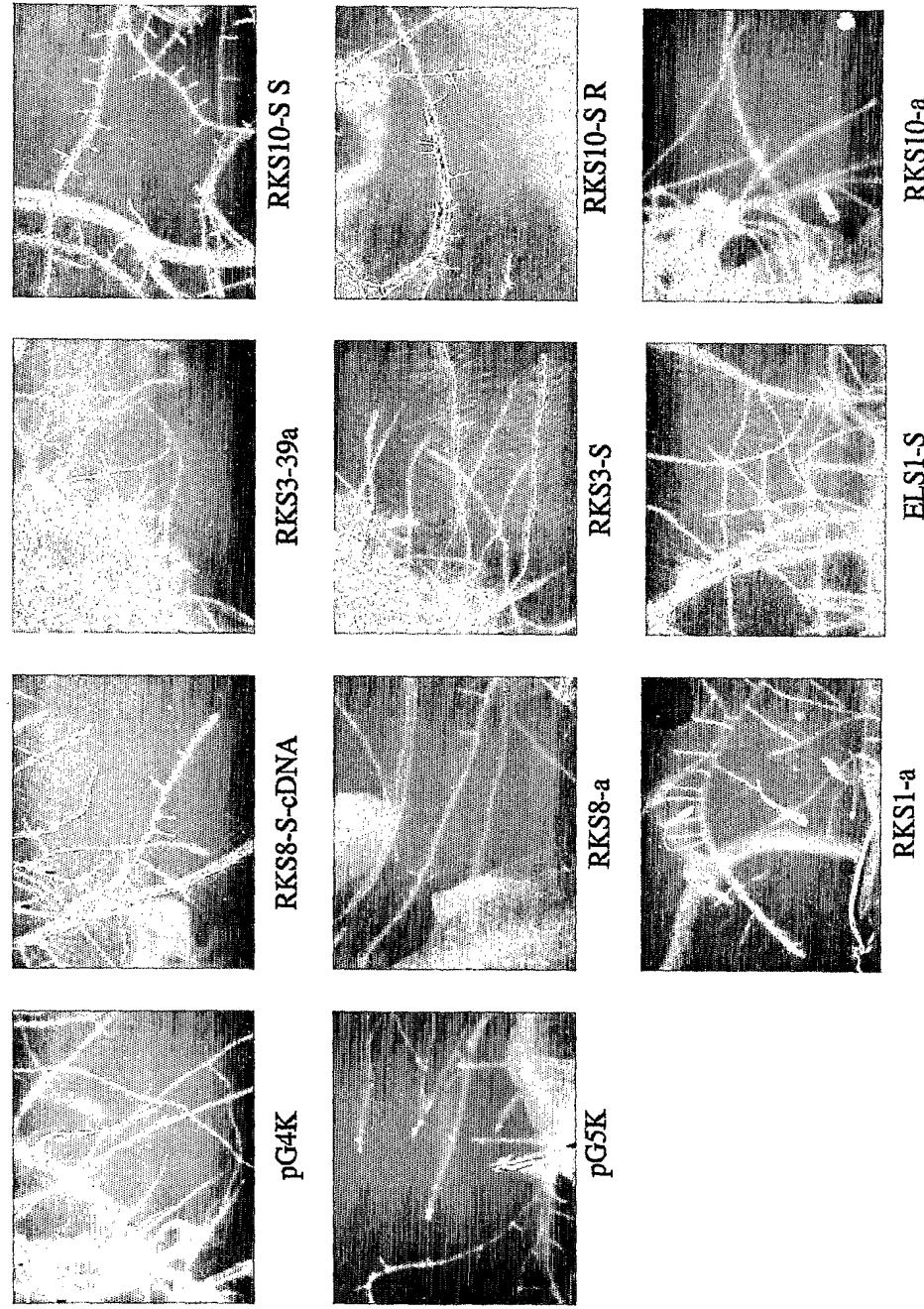


Fig. 27

Root cells of transgenic
Arabidopsis thaliana

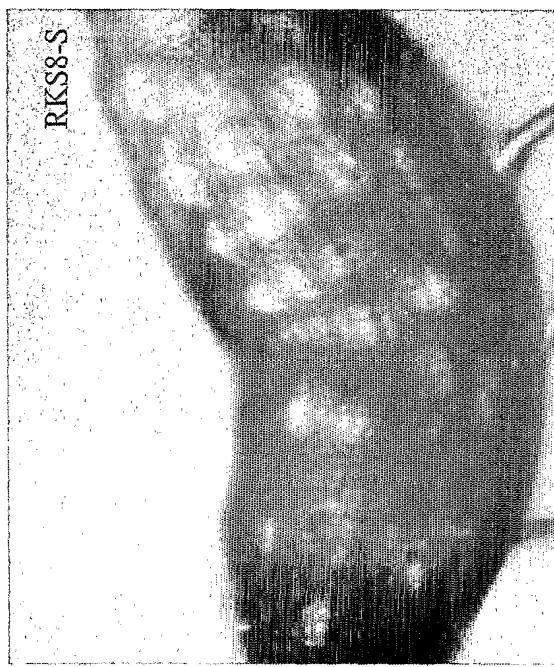
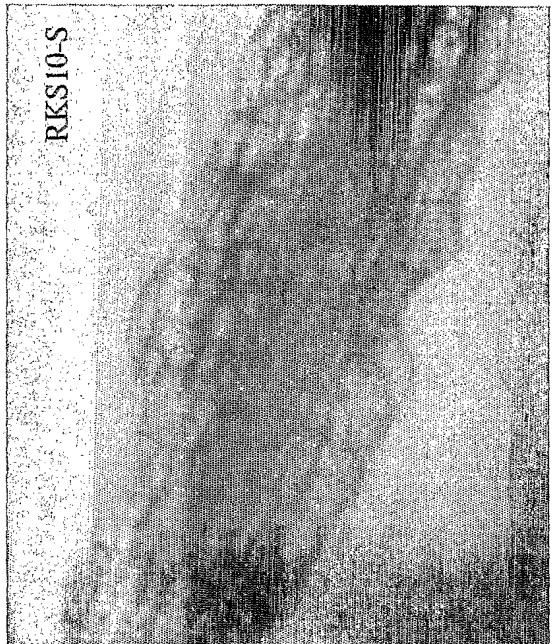


Fig. 28

Influorescences of T1 transgenic
Arabidopsis WS plants



Fig. 29

Influorescences of T1 transgenic
Arabidopsis WS plants

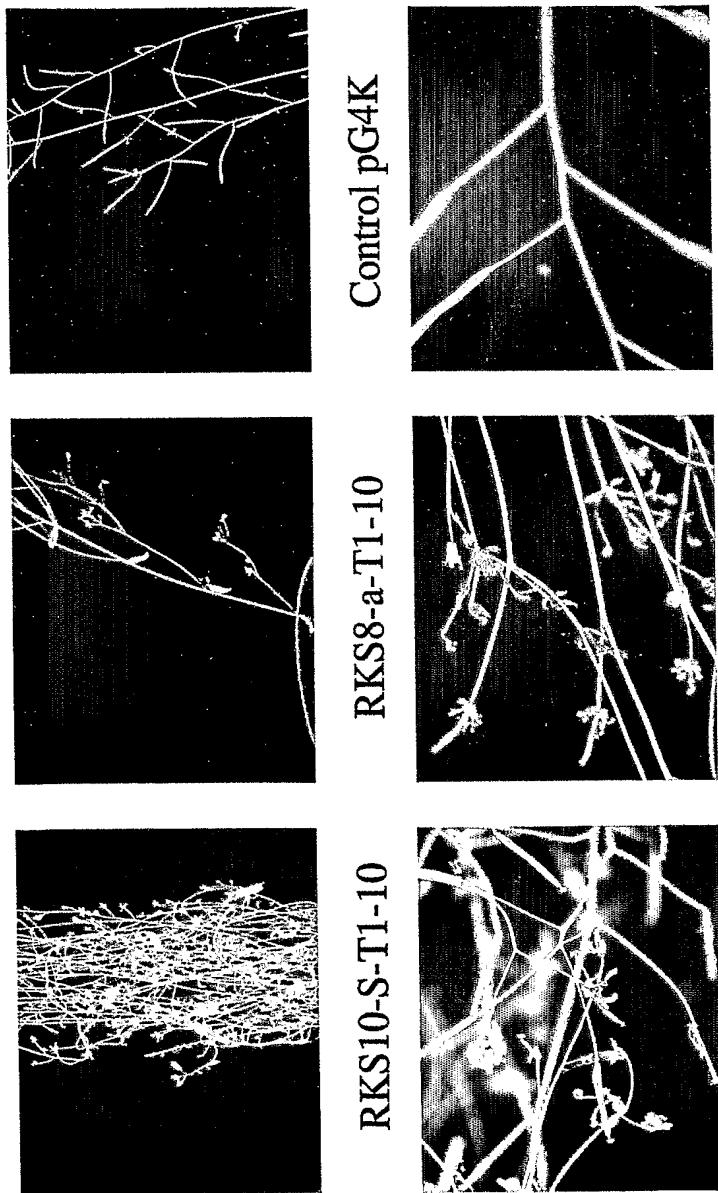


Fig. 30

RKS10a T1 expression constructs in
Arabidopsis thaliana

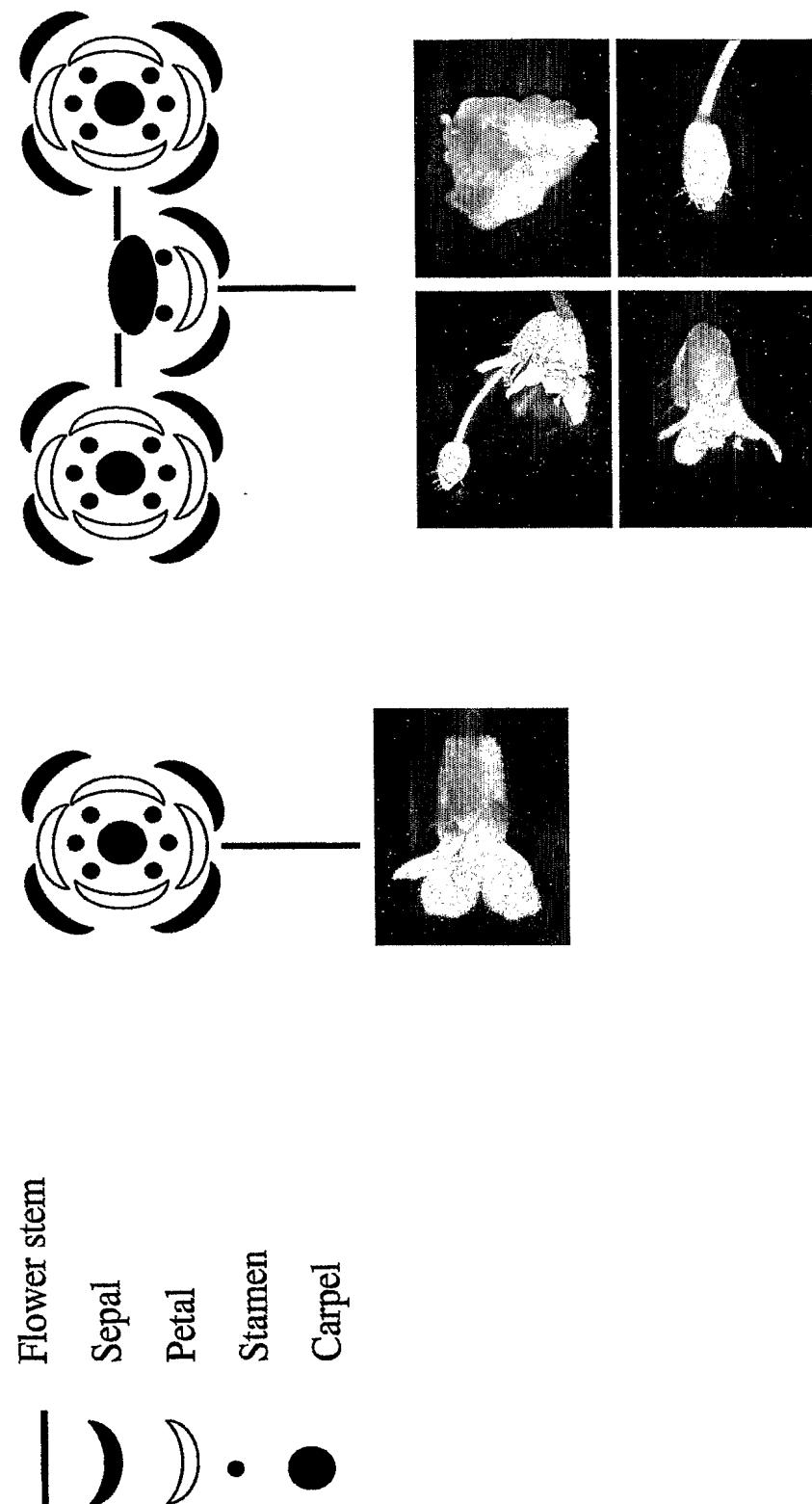


Fig. 31

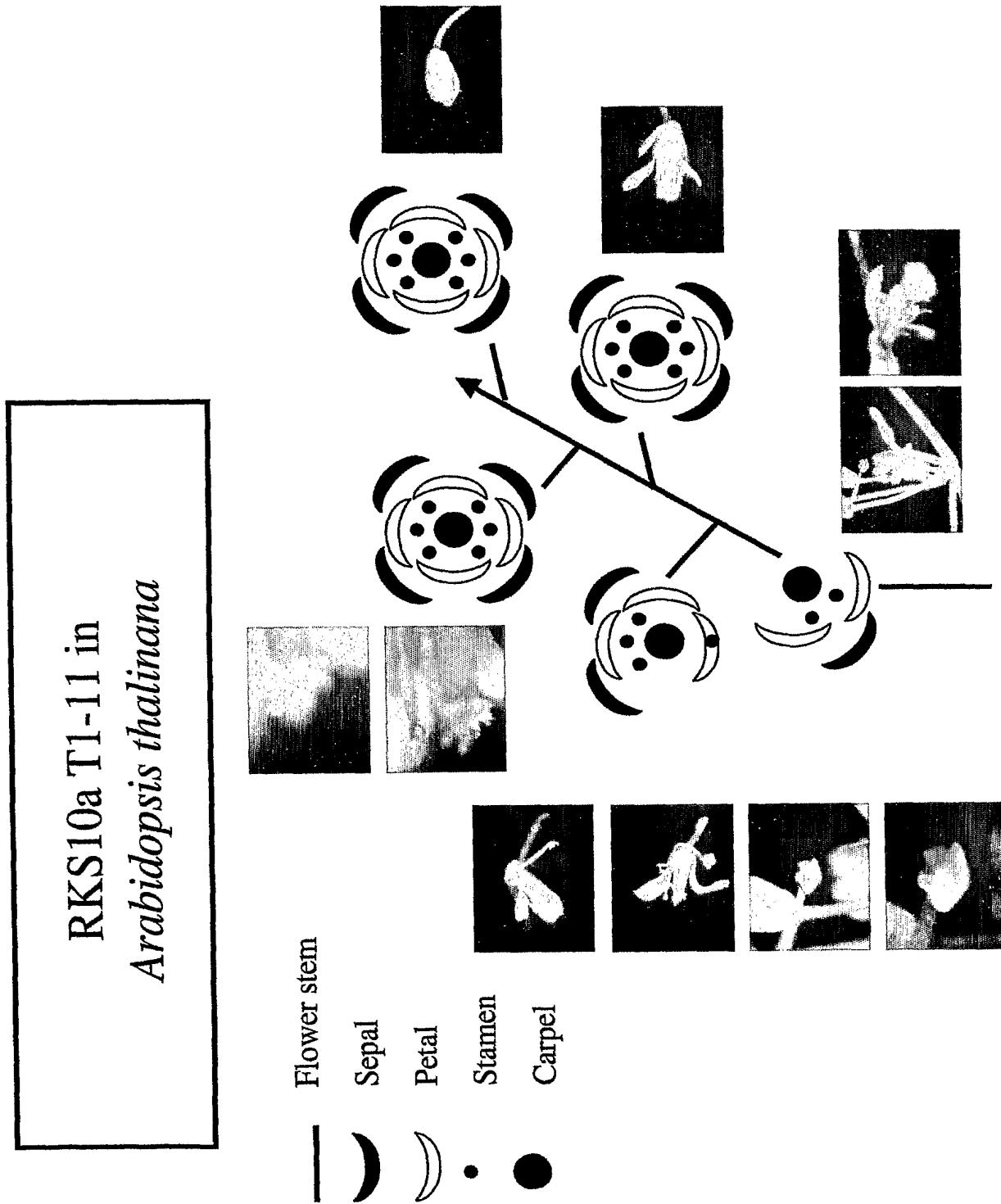
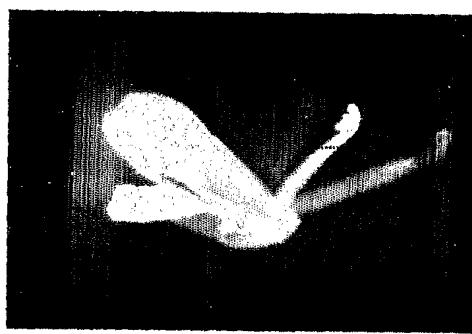


Fig. 32

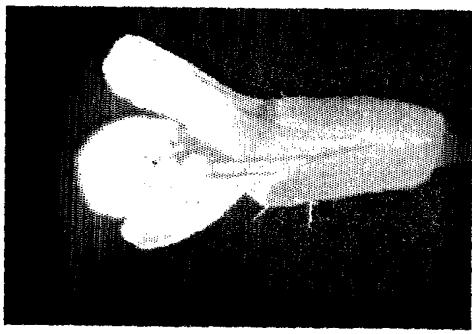
RKS10 antisense effects in
Arabidopsis thaliana



detail flower RKS10a T1-11



RKS10a T1-11



pGreen 4K

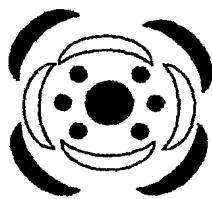


Fig. 33

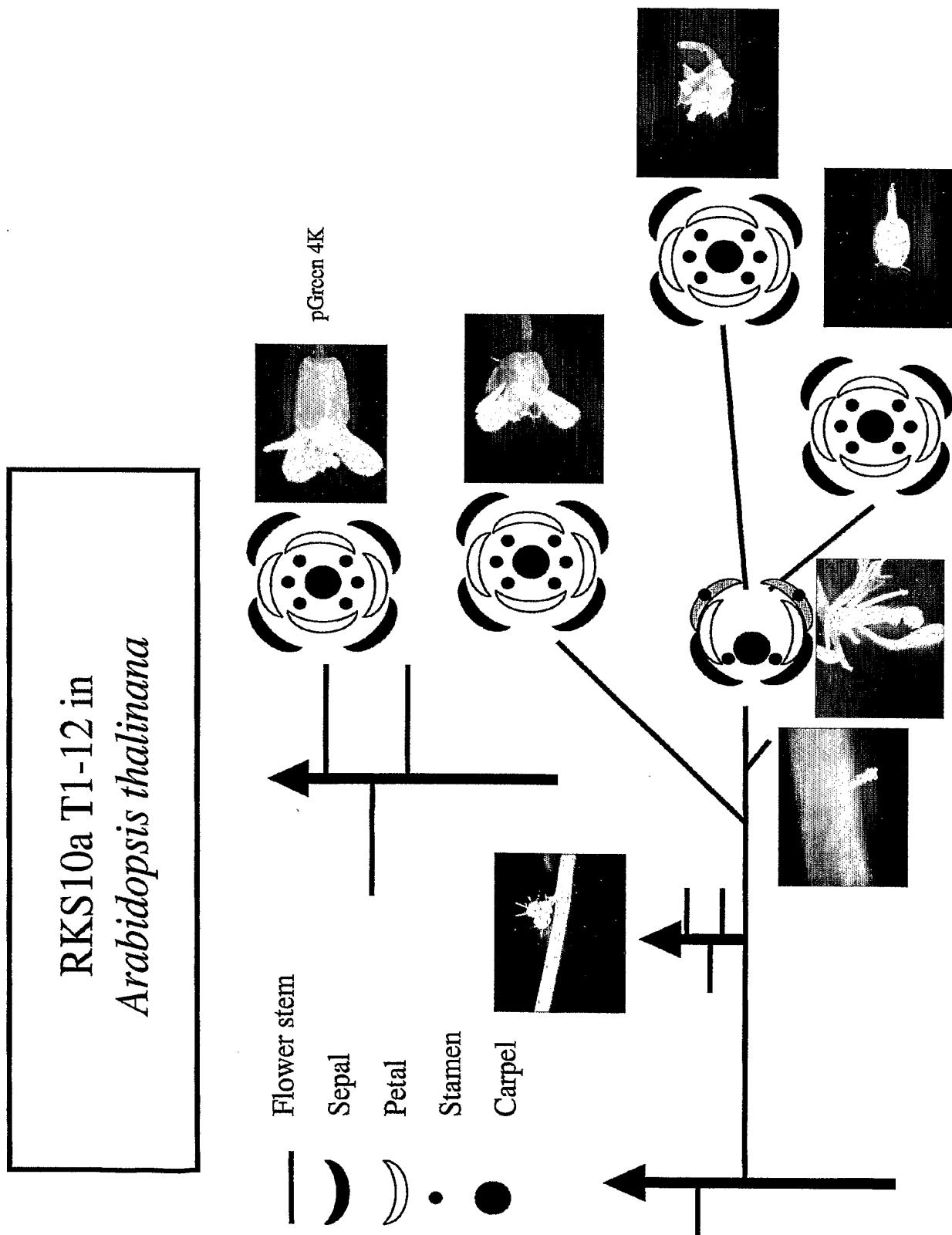


Fig. 34

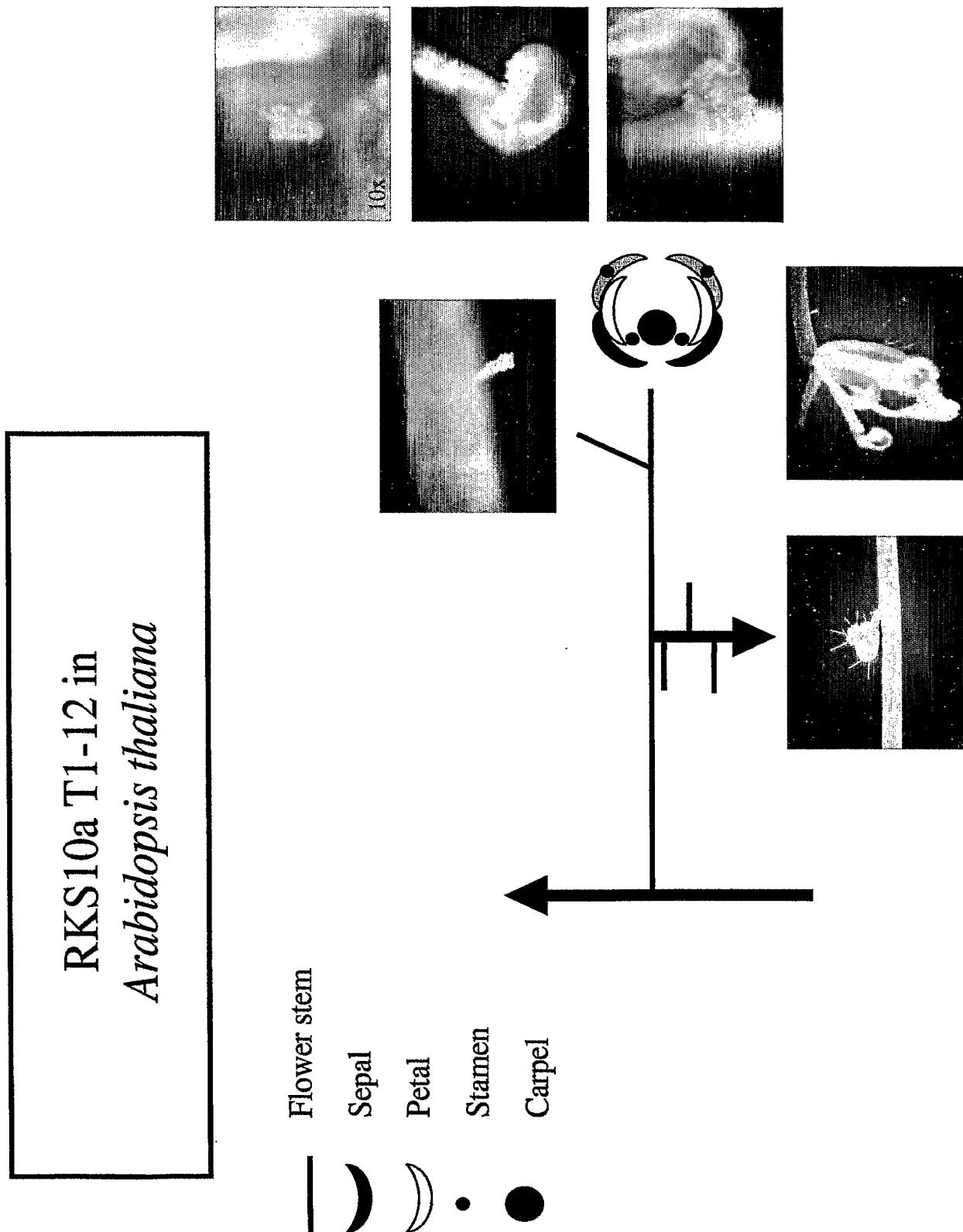


Fig. 35

RKS13 regulates
flower meristem identity in
Arabidopsis thaliana

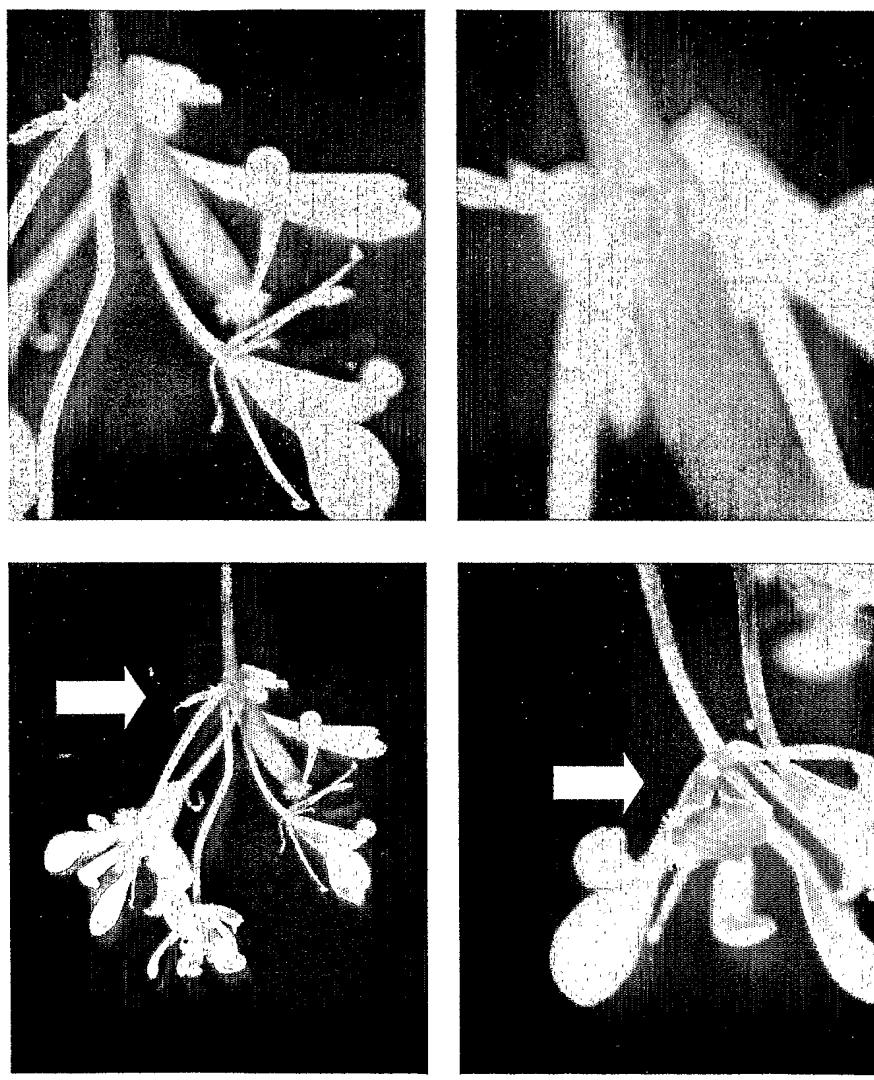
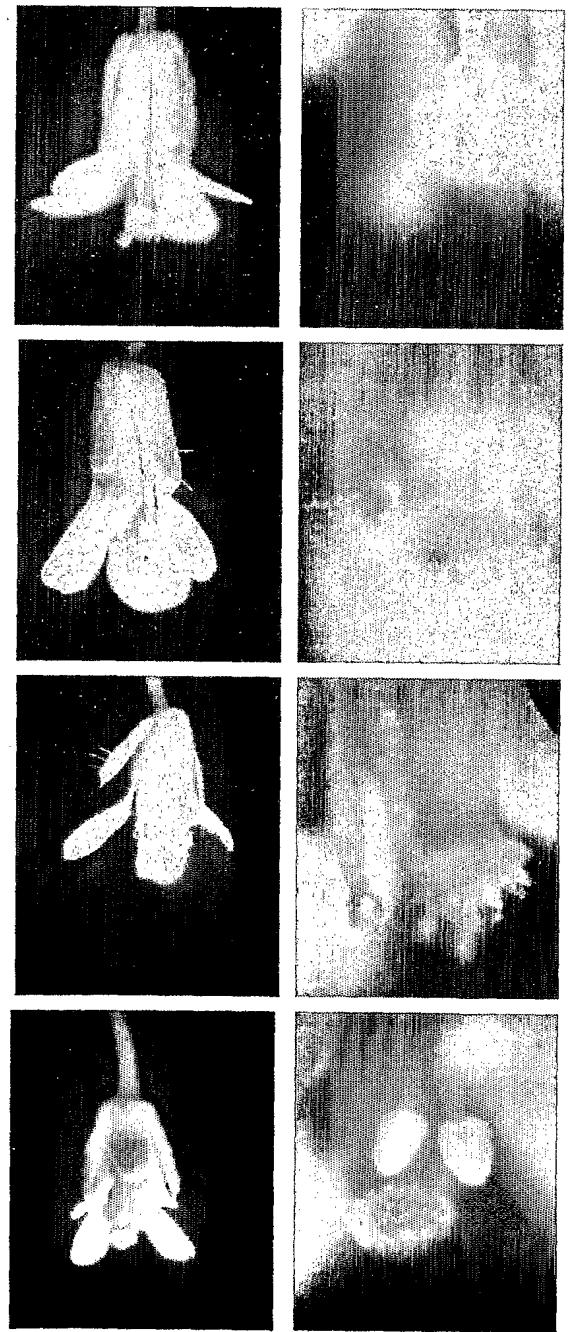


Fig. 36

Male sterile transgenes in
Arabidopsis thaliana



RKS10S T1-10 RKS10a T1-11 pGreen4K
no pollen formed almost no pollen normal pollen
ELS 2 157.21S T1-11 T2-2 pollen development aborted